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(54) Title: LIPOSOMAL PRODRUGS COMPRISING DERIVATIVES OF CAMPTOTHECIN AND METHODS OF TREATING CANCER USING THESE PRODRUGS

Mouse CPT Plasma Levels

2 MG/KG, I.S.
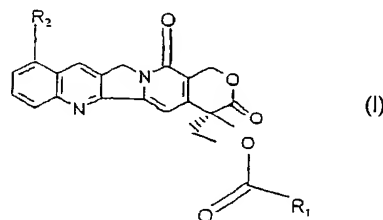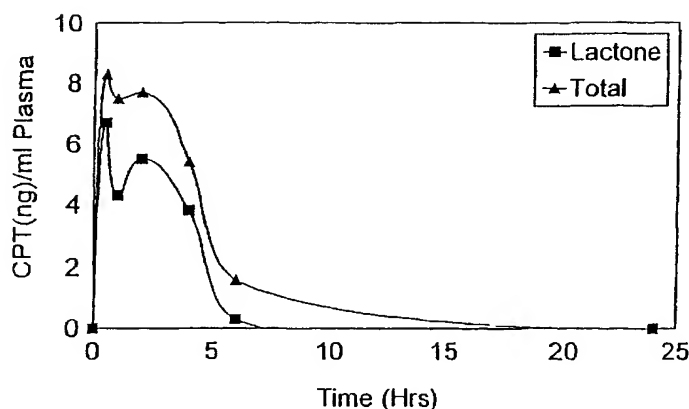
(57) Abstract: Derivatives of camptothecin as represented by general formula (I) are described, wherein when $R_2$ is H, $R_1$ is a $C_2$-$C_4$ alkyl group, a $C_6$-$C_{15}$ alkyl group, a $C_3$-$C_8$ cycloalkyl group, a $C_2$-$C_{15}$ alkenyl group or a $C_2$-$C_{15}$ epoxy group; and when $R_2$ is a nitro group or an amino group, $R_1$ is a $C_1$-$C_{15}$ alkyl group, a $C_2$-$C_{15}$ alkenyl group, a $C_3$-$C_8$ cycloalkyl group, or an epoxy group. Liposomal prodrugs including these specific derivatives of camptothecin restrained by a liposomal delivery system are also described. Processes for making these prodrugs and for using them in cancer treatment are also disclosed.

# LIPOSOMAL PRODRUGS COMPRISING DERIVATIVES OF CAMPTOTHECIN AND METHODS OF TREATING CANCER USING THESE PRODRUGS

5          This application is a continuation-in-part of U.S. Patent Application No. 09/365,632,

filed August 3, 1999.

## FIELD OF THE INVENTION

The present invention is directed to derivatives of camptothecin and is also directed

10    to compositions including derivatives of camptothecin in delivery systems, preferably having

low toxicity. The present invention also relates to the use of these derivatives for cancer

treatment. The disclosures of all documents referred to in this application are incorporated

herein in whole by reference.

15    ## BACKGROUND OF THE INVENTION

Camptothecin, a cytotoxic alkaloid first isolated from the wood and bark of

*Camptotheca Acuminata (Nyssaceae)* by Wall and his coworkers *(J. Am. Chem. Soc. 88,*

*3888, 1966),* was shown to have antitumor activity against the mouse leukemia L 1210

system. The structure of camptothecin, an alkaloid which has a commonly occurring indole

20    alkaloid group (Heckendorf et al, *J Org. Chem. 41, 2045, 1976),* is shown below as

Formula (X).

(X)
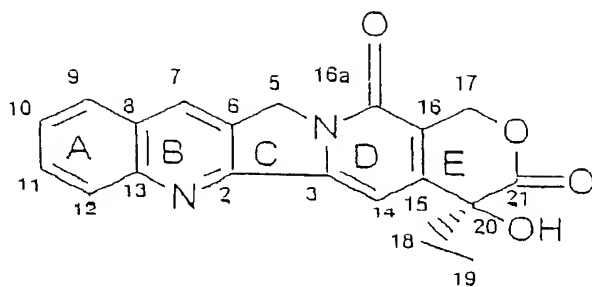


This compound has a pentacyclic ring system with only one asymmetrical center in ring E with a 20(S)-configuration. The pentacyclic ring system includes a pyrrolo [3, 4 - b] quinoline moiety (rings A, B and C), a conjugated pyridone (ring D), and a six-membered lactone (ring E) with an $\alpha$ - hydroxyl group. Camptothecin was of great interest from the time of its initial isolation due to its noteworthy activity in the mouse leukemia L 1210 system. Earlier data for the antitumor activity of camptothecin were obtained by employing experimentally transplanted malignancies such as leukemia L 1210 in mice, or Walker 256 tumor in rats *(Chem. Rev. 23, 385, 1973, Cancer Treat. Rep. 60, 1007, 1967)*. Subsequent clinical studies showed that this compound was not usable as an anticancer agent *in vivo* due to its high toxicity. Camptothecin itself is insoluble in water. Therefore, camptothecin was evaluated clinically as a water-soluble sodium carboxylate salt in the early times. This form of camptothecin produced severe toxicity and seemed devoid of anticancer activity (Gottlieb et al, *Cancer Chemother. Rep. 54, 461, 1970,* and *56, 103, 1972,* Muggia et al, *Cancer Chemother. Rep. 56, 515, 1972,* Moertel et al, *Cancer Chemother. Rep. 56, 95, 1972,* and Schaeppi et al, *Cancer Chemother. Rep. 5:25, 1974)*. These results caused the discontinuation of phase II trials. Continued evaluation of this agent showed that the sodium carboxylate salt is only 10% as potent as the native camptothecin with the closed lactone ring intact (Wall et al, *In International Symposium on Biochemistry And Physiology of The Alkaloids, Mothes et*

*al, eds, Academie - Verlag, Berlin, 77, 1969,* Giovanella et al, *Cancer res. 51, 3052, 1991).*

In addition, important parameters for antitumor activity in the camptothecin family have been established (Wall et al, *Ann. Rev., Pharmacol. Toxicol. 17, 117, 1977).* These results indicate that intact lactone ring E and $\alpha$ - hydroxyl group are essential for antitumor activity.

In 1989, Giovanella et al. found that some of the non-water soluble derivatives of camptothecin have high antitumor activity against xenograft of human tumors (Giovanella et al., *Science, 246, 1046, 1989).* It has also been shown that administration of camptothecin with closed lactone ring is superior to injections of water-soluble carboxylate salt (Giovanella et al, *Cancer Res.,* 51, 3052, 1991). These findings further confirmed the importance of the intact lactone ring.

Clearly, there is a need to modify 20(S)-camptothecin ("CPT") to enable the lactone form to stay longer in the body while retaining the structural elements (i.e. 20-hydroxyl and lactone ring E) which are essential for its antitumor activity.

Ring opening of CPT leads to much more potent anticancer activity in mice than in humans. In effect, CPT administered intramuscularly ("i.m."), subcutaneously ("s.c."), and intrastomach ("i.s.") has proved to be a very potent anticancer agent against human tumors in mice, *i.e.,* when growing as xenotransplants in nude mice (Giovanella *et al,* Cancer Res. 51:3052, 1991). However, when tumors were treated with CPT in humans, a lower degree of anticancer activity in humans, than in mice, was exhibited (Stehlin *et al.,* In Camptothecins: New Anticancer Agents, 1995, CRC Press, pp. 59-65).

The same phenomenon was observed with other CPT-derivatives. In mice, 9-nitrocamptothecin ("9NC") has proven to be 2-3 times more potent than CPT against human tumor xenografts causing the total eradication of all the human malignancies treated (Pantazis *et al.,* Cancer Res. 53:1577, 1993; Pantazis *et al.,* Int. J. Cancer 53:863, 1995).

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Pharmacological studies demonstrated that the majority (57%) of the 9NC drug

present in the plasma after i.s. administration is in the closed lactone form (Fig. 3).

Pharmacological studies on the plasma levels of 9NC after oral administration to Phase I

clinical trial patients demonstrate that, on average, only ~ 3% of the drug present is in the

5    closed lactone form (Figs. 4 and 5).

In perfect agreement with such findings, the clinical responses in this group of

patients, although higher than those obtained with CPT are still a far cry below the results

obtained in mice (32/32 complete tumor regressions in mice versus 2/32 in humans). Clearly,

again there is a pressing need for a modification which will slow and delay the lactone ring

10   opening upon its entrance into the blood circulation.

A number of attempts have been made to provide more active derivatives of

camptothecin and longer lasting forms. U.S. Patent Application No. 09/030,357 to Cao et

al. provides CPT-derivatives which are effective antitumor agents. In addition, Cao et al.

describe in the application new active CPT-derivatives which sustain the opening of the

15   lactone ring E, which makes the antitumor activity last longer than its mother analog, CPT.

Unfortunately, because a mammalian body breaks down CPT-derivatives over time by

opening the lactone ring, a need still exists for a CPT-derivative delivery system that keeps

the CPT-derivative intact even longer in the body, thus prolonging the antitumor activity of

the derivative.

## SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a new liposomal delivery system for CPT-derivatives which enables the derivatives to remain intact longer in a mammalian body, particularly in a human body.

5          It is another object of the present invention to provide a new liposomal delivery system for water insoluble CPT-derivatives possessing good absorbability in the living body.

It is still another object of the present invention to provide a new liposomal delivery system for CPT-derivatives which retains the lactone ring E and the 20-hydroxyl group intact, which is important for antitumor activity.

10          It is still a further object of the present invention to provide a method for preparing CPT-derivatives, preferably water insoluble derivatives, in a liposomal delivery system.

Additional objects and advantages of the present invention will be set forth in part in the description which follows, and in part will be apparent from the description, or may be learned by practice of the present invention. The objects and advantages of the present

15          invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

To achieve the objects and in accordance with the purpose of the present invention, as embodied and broadly described herein, the present invention relates to a compound of the formula:
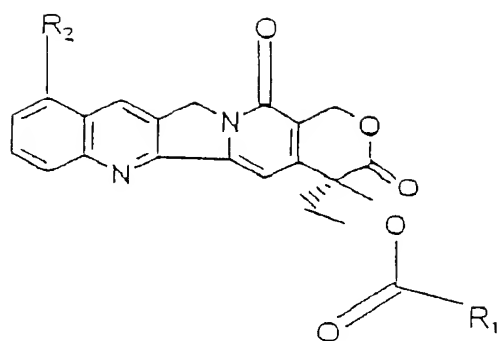
wherein $R_2$ is H, $NH_2$, or $NO_2$ and $R_1$ is a $C_2$-$C_4$ alkyl group, a $C_6$-$C_{15}$ alkyl group, a $C_3$-$C_8$ cycloalkyl group, a $C_2$-$C_{15}$ alkenyl group, or an epoxy group when $R_2$ is H; and $R_1$ is a $C_1$-$C_{15}$ alkyl group, a $C_1$-$C_{15}$ alkenyl group, a $C_3$-$C_8$ cycloalkyl group, or an epoxy group when $R_2$

is $NO_2$.

The invention also relates to a method for treating cancer and/or malignant tumors in a mammal and comprises administering an effective amount of one or more of the above CPT-derivatives in a liposomal delivery system.


## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph showing the presence of CPT in the closed lactone form in mice after intrastomach administration.

Fig. 2 is a graph showing the amount of CPT and its closed lactone form in a human after oral administration.

Fig. 3 is a graph of the amount of 9-nitrocamptothecin and its closed lactone ring form in a mouse after oral administration.

Figs. 4 and 5 show the amount of 9-nitrocamptothecin and its closed lactone ring in clinical trial patients who received the dosage by oral administration.

Fig. 6 is a graph representing the antitumor activity of camptothecin 20(S) propionate in an implanted human breast carcinoma CLO in nude mice.

Fig. 7 is a graph showing the presence of camptothecin 20(S) propionate and its closed lactone ring form in human plasma after oral administration.

Fig. 8 is a graph showing the presence of 9-nitrocamptothecin-20-O-propionate in a patient who received the compound orally.

Fig. 9 is a graph which shows the results obtained for SW48 colon carcinoma in nude mice treated 5 times a week with a CPT-derivative of the present invention at the dosage of 20 mg/kg by intrastomach means.

Fig. 10 is a graph which shows the results obtained for BRO melanoma in nude mice treated with a CPT-derivative of the present invention at the dosage of 20 mg/kg by intrastomach means.

Fig. 11 is a graph which shows the results obtained for BRE stomach carcinoma in nude mice treated with a CPT-derivative of the present invention at a dosage of 10 and 20 mg/kg respectively by intrastomach means.

Fig. 12 is a graph which shows the results obtained for DOY lung carcinoma in nude mice treated with CPT-derivative of the present invention at a dosage of 10 and 20 mg/kg respectively by intrastomach means.

Fig. 13 is a graph which shows the results obtained for 2774 ovarian carcinoma in nude mice treated with a CPT-derivative of the present invention at a dosage of 10 and 20 mg/kg respectively by intrastomach means.

Fig 14. is a graph which shows the results obtained for McC colon carcinoma in nude mice treated with a CPT-derivative of the present invention at a dosage of 20 mg/kg by intrastomach or intramuscular means.

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Fig. 15 is a graph which shows the results obtained for SQU colon carcinoma in nude mice treated with a CPT-derivative of the present invention at a dosage of 10 mg/kg by intrastomach or intramuscular means.

Fig. 16 is a graph which shows the results obtained for CLO breast carcinoma in nude mice treated with a CPT-derivative of the present invention at a dosage of 8 mg/kg by intramuscular means.

Fig. 17 sets forth the chemical structures of several CPT-derivatives used in experiments.

Fig. 18 shows the results of flow cytometry indicating treatment time, cell number, and DNA content for 20(S) CPT and CPT-derivatives.

Figs. 19a and 19b also show a flow cytometry analysis for 20(S) CPT and CPT-derivatives reflecting treatment time, cell number, and DNA content.

Fig. 20 is a set of graphs showing the results obtained for breast carcinoma in nude mice treated with CPT-derivatives by intrastomach means.

## DETAILED DESCRIPTION OF THE INVENTION

Metabolism studies of camptothecin in human plasma carried out in the laboratory showed that the only metabolite detected is the ring-opened sodium carboxylate salt which is toxic and inactive.  The measurement of pharmacokinetics for CPT in human plasma indicates that the half-life time of the drug with lactone intact is 30 min.  These results imply that the drug will lose 90% of its activity and produce a lot of toxicities in a very short time after a patient takes it.

Comparative pharmacological studies in mice and humans have demonstrated that in mice the majority of the CPT present in the plasma after intrastomach administration is of the

closed lactone form (Fig. 1), approximately 54% of the area under the curve. In humans, on the contrary, only about 0.4% of the area under the curve after oral administration of CPT is in the form of closed ring lactone (Fig. 2).

This difference between a mouse and a human is caused by the fact that although the blood pH of the mouse and human are the same, *i.e.*, 7.4, the human albumin, which catalyzes the conversion of CPT into its sodium salt is ~ 100 times more efficient in this process than mouse albumin (Mi and Burke, Biochem. 33:12540, 1994).

According to the present invention, CPT is converted into more lipo-soluble molecules, hereinafter, also referred to as "prodrugs." The prodrugs are then incorporated or encapsulated in, surrounded or entrapped by, or otherwise restrained by a liposomal delivery system to form the liposomal prodrugs of the present invention. When taken orally by patients, the liposomal prodrugs are rapidly introduced into the bloodstream of a patient and readily converted to the parent compound in the body.

Conversion of the prodrugs to the mother compound, CPT, is mediated by a group of enzymes called esterases present in the blood of many animals, including humans. Since the prodrugs are rapidly distributed throughout the body in a short period of time after delivery, these compounds exist at a very low concentration at the time they undergo enzymatic hydrolysis by which the mother camptothecin is released, which prevents CPT from precipitating in the bloodstream.

In an attempt to synthesize new CPT-derivatives and delivery systems therefor with extremely reduced toxicity, while maintaining the inherent antitumor activity, the present inventors have performed an acylation reaction of camptothecin with various organic acids, organic acid chlorides, and organic acid anhydrides. A number of new camptothecin derivatives have been obtained and are described in detail in U.S. Patent No. 5,968,943 and

PCT/US97/01728, which are incorporated herein in their entireties by reference. They are

characterized by formula I shown below:
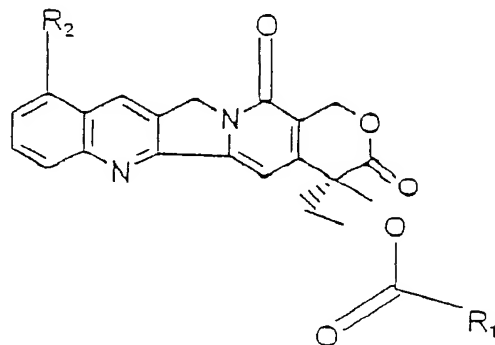


5    wherein $R_2$ is H, $NH_2$, or $NO_2$. $R_1$ in formula I represents a $C_2$-$C_4$ alkyl group, a $C_6$-$C_{15}$ alkyl

group, a $C_3$-$C_8$ cycloalkyl group, a $C_2$-$C_{15}$ alkenyl group or a $C_2$-$C_{15}$ epoxy group when $R_2$ is

H. When $R_2$ is $NO_2$, or $NH_2$, $R_1$ is a $C_1$-$C_{15}$ alkyl group, a $C_3$-$C_8$ cycloalkyl group, a $C_2$-$C_{15}$

alkenyl group or a $C_2$-$C_{15}$ epoxy group. Preferably, when $R_2$ is H, $R_1$ is $CH_2CH_3$;

$CH_2CH_2CH_3$; $CH_2CH_2CH_2CH_3$; $CH_2CH_2CH_2CH_2CH_2CH_3$; $CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$;

10    $CH=CH_2$; $CH=CHCH_3$ (trans); or



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$$CH \underset{CH_2 \qquad CH_2}{\overset{CH_2 \qquad CH_2}{\phantom{X}}} CH_2 \quad ;$$

$$\overset{O}{\underset{CH \qquad CH_2}{\triangle}} \quad ; \text{ or}$$
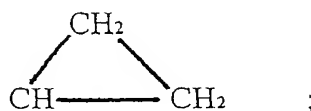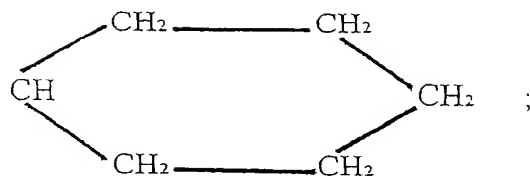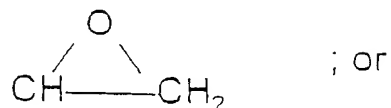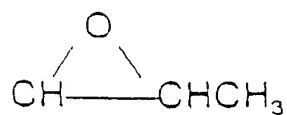
$$\overset{O}{\underset{CH \qquad CHCH_3}{\triangle}}$$

Also, when $R_2$ is $NH_2$ or $NO_2$, $R_1$ is $NH_2$ or preferably $CH_3$; $CH_2CH_3$; or $CH_2CH_2CH_3$;

$CH=CH_3$; $CH=CHCH_3$(trans); $CH_3$; $CH_2CH_3$; or $CH_2CH_2CH_3$; $CH=CH_3$;

$CH=CHCH_3$(trans); or

$$\overset{CH_2}{\underset{CH \qquad CH_2}{\triangle}} \quad ;$$

$$CH \underset{CH_2 \qquad CH_2}{\overset{CH_2 \qquad CH_2}{\phantom{X}}} CH_2 \quad ;$$

$$\overset{O}{\underset{CH \qquad CH_2}{\triangle}} \quad ; \text{ or}$$

$$\overset{O}{\underset{CH \qquad CHCH_3}{\triangle}}$$

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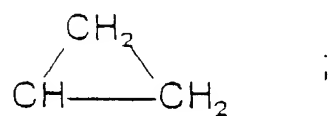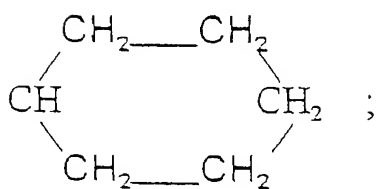
The analogues of 9-nitrocamptothecins are prepared by the acylation of 9-nitrocamptothecin with organic anhydrides. All epoxy derivatives can be prepared by the reactions of the corresponding alkenyl esters of the present invention with m-chloroperoxy benzoic acid in benzene at room temperature.

5          The preferred derivatives display significant antitumor activity with much lower toxicity than their parent camptothecin, CPT. The animal experimental data for these new derivatives were collected. Camptothecin 20(S) propionate (where $R_1$ is ethyl and $R_2$ is H) is designated as Prodrug 2 and taken as a example to disclose some in vivo experimental data. Fig. 6 represents the antitumor activity of this compound in different doses against the

10         implanted human breast carcinoma (CLO) in nude mice. Table 1 represents the toxicity of this compound against nude mice with different doses. The change of body weight of mice is recorded with the time. There were no losses of mice body weights during the test time period.
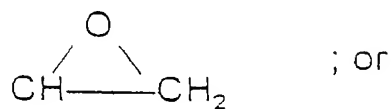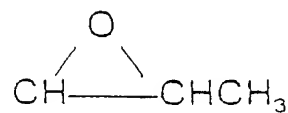
Table 1
The Changes of Body Weights of Mice during The Test Time Period

| Dose (mg/kg) | Time (days) / Body weight (gms) | | | | | |
|---|---|---|---|---|---|---|
| Control | 21 / 32.7 | 27 / 33.5 | 34 / 34.4 | 41 / 35.2 | 48 / 35.0 | 56 / 36.7 |
| 5 | 21 / 33 | 27 / 33.7 | 34 / 34.3 | 41 / 33.5 | 48 / 32.9 | 56 / 33.2 |
| 6 | 21 / 32.9 | 27 / 33.4 | 34 / 33.8 | 41 / 33.5 | 48 / 34.0 | 56 / 32.2 |
| 7 | 21 / 30.8 | 27 / 31.7 | 34 / 30.6 | 41 / 31.1 | 48 / 31.6 | 56 / 31.5 |
| 8 | 21 / 32.9 | 27 / 34.1 | 34 / 34.0 | 41 / 33.4 | 48 / 33.9 | 56 / 33.2 |

15         The measurement of pharmacokinetics for Prodrug 2, camptothecin 20(S) propionate, shows that the lactone ring remained intact in human plasma much longer than its mother camptothecin, CPT. Table 2 represents this result.

Table 2
Comparison of Lactone % of 20(S) Propionate and
Lactone % of Camptothecin in Human Plasma

| Time (Hr.) | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|
| Lactone % for prodrug 2 | 100 | 86 | 77 | 68 | 56 |
| Lactone % for camptothecin | 100 | 12 | 0.5 | 0 | 0 |

As reflected in the tables, augmenting the biological life of the closed lactone ring has been achieved. As shown in Fig. 2, the % of CPT present as closed lactone form in human blood after oral administration is 0.4%. Its analogue, 20-(S) propionate, under similar conditions reaches 2.8% (Fig. 7), an increase of sevenfold. This compound exhibits antitumor activity against human tumor xenografts and an exceptional lack of toxicity, even at enormous doses in mice. Even more striking are the results obtained with 9NC. As shown in Figs. 4 and 5, after oral administration of this compound, only ~ 3% of it is present in the plasma as lactone. When its analogue, Prodrug 11 of the present invention, (where $R_1$ is ethyl and $R_2$ is $NO_2$) was prepared and administered orally to a patient, the lactone form constituted 68.7% of the total, an increase of more than twentyfold (Fig. 8). This compound also exhibited antitumor activity against xenografts of human tumors in nude mice, even higher than the camptothecin analogue, Prodrug 2, where $R_1$ is ethyl and $R_2$ is H and with very low toxicity.

To even further provide a long lasting antitumor activity, the prodrugs are restrained in, on or by a liposomal delivery system. The system restrains the prodrug by incorporating, encapsulating, surrounding, or entrapping the prodrug in, on, or by lipid vesicles or liposomes, or by micelles.

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Preferably, the prodrug is solubilized in liposomes. The liposomes may include, for example, lipids such as cholesterol, phospholipids, or micelles comprised of surfactant such as, for example, sodium dodecylsulfate, octylphenolpolyoxyethylene glycol, or sorbitan mono-oleate. Typically, the prodrugs bind to the lipid bilayer membrane of the liposome with high affinity. The liposome bound prodrug can preferably intercalate between the acyl chains of the lipid. The lactone ring of the camptothecin-derivative, membrane-bound prodrug is thereby removed from the aqueous environment inside and outside of the liposome and thus protected from hydrolysis. Since the liposome-bound drug is protected from hydrolysis, the antitumor activity of the drug is preserved. For the camptothecin prodrugs which have a lower affinity for the liposome membrane and thus disassociate from the liposome membrane to reside in the interior of liposome, the pH of the interior of the liposomes may be reduced thereby preventing hydrolysis of such camptothecin-derivative prodrugs.

A preferred group of liposomal delivery systems which may be used in accordance with the present invention include those described in U.S. Patent Nos. 5,552,156 and 5,736,156, which are herein incorporated in their entireties by reference. Other liposomal delivery systems which may be employed in accordance with the present invention include liposomes containing active agents aggregated with lipids or surfactants as described in U.S. Patent Nos. 5,827,533 and 5,882,679; lipid vesicles formed with alkyl ammonium fatty acid salts as described in U.S. Patent No. 5,874,105; liposomes for encapsulating active agent dry powder compositions as described in U.S. Patent No. 5,783,211; liposomal drug delivery systems for topical patches as described in U.S. Patent No. 5,718,914; the liposomes described in U.S. Patent No. 5,631,237; the liposome and lipid complex compositions described in U.S. Patent Nos. 5,549,910 and 5,077,057; the liposomes used for sustained release of steirodial drugs as described in U.S. Patent No. 5,043,165; the liposomes

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described in U.S. Patent No. 5,013,556; and the liposomes described in U.S. Patent No. 4,663,161; all of which are herein incorporated in their entireties by reference.

Unilamellar liposomes, also referred to as single lamellar vesicles, are spherical vesicles comprised of one lipid bilayer membrane which defines a closed compartment. The
5    bilayer membrane is composed of two layers of lipids; an inner layer and an outer layer. The outer layer of lipid molecules are oriented with their hydrophilic head portions toward the external aqueous environment and their hydrophobic tails pointed downward toward interior of the liposome. The inner layer of lipid lays directly beneath the outer layer; the lipids are oriented with their heads facing the aqueous interior of the liposome and their tails toward
10   the tails of outer layer of lipid.

Multilamellar liposomes, also referred to as multilamellar vesicles are composed of more than one lipid bilayer membrane, which membranes define more than one closed compartment. The membranes are concentrically arranged so that the different membranes are separated by compartments much like an onion skin.

15         As used herein the phrase "liposomal prodrugs" means either that some or all of the camptothecin-derivative prodrug is located in one or more of the compartments of a liposome or micelle, or the camptothecin-derivative prodrug is bound to the membrane of the liposome. Preferably, the delivery systems a liposome comprising a lipid bilayer membrane which entraps a camptothecin-derivative prodrug.  As used herein, the phrase "bound to lipid
20   membrane" means that at least the lactone ring of some or all of the camptothecin-derivative prodrug binds to the lipid membrane of the liposome, and where the liposome contains more than one bilayer membrane the camptothecin-derivative prodrug is bound to at least 1 membrane. Those camptothecin-derivative prodrugs that have a high affinity for such membrane tend to remain bound to the membrane. Those camptothecin-derivative prodrugs

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with a low affinity for the liposome membrane, will at least partially disassociate from the liposome membrane and reside in the liposome compartment.

Micelles as defined herein are spherical receptacles comprised of a single, monolayer membrane which defines a closed compartment and the membrane is comprised of surfactant molecules oriented so that the hydrocarbon tails are oriented toward the compartment and the polar head portions are oriented toward the external aqueous environment. The camptothecin-derivative prodrugs, when associated with micelles, are either in the compartment, bound to the micelle membrane, or bound to the outside surface of the micelle.

Liposomes have been used successfully to administer medications to cancer patients, and have been shown to be useful clinically in the delivery of anticancer drugs such as doxorubicin, daunorubicin, and cisplatinum complexes. Forssen, et al., Cancer Res. 1992, 52: 3255-3261; Perez-Soler, et al. Cancer Res. 1990, 50: 4260-4266; and, Khokhar, et al. J. Med. Chem. 1991, 34: 325-329, all of which are incorporated herein in their entireties by reference.

Similarly, micelles have also been used to deliver medications to patients, (Brodin et al., Acta Pharm. Suec. 19 267-284 (1982)) and micelles have been used as drug carriers and for targeted drug delivery, (D. D. Lasic, Nature 335: 279-280 (1992); and, Supersaxo et al., Pharm. Res. 8: 1286-1291 (1991)), including cancer medications, (Fung et al., Biomater. Artif. Cells. Artif. Organs 16: 439 et. seq. (1988); and Yokoyama et al., Cancer Res. 51: 3229-3236 (1991)), all of which are incorporated herein in their entireties by reference.

The liposomes and/or micelles of the present invention containing the camptothecin-derivative prodrugs can be administered to a cancer patient, typically intravenously. The liposomes and/or micelles are carried by the circulatory system to the cancer cells where the membrane of the vesicle fuses to the membrane of the cancer cell thereby releasing the

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camptothecin-derivative prodrug to the cancer cell, or where the liposomes and/or micelles remain adjacent to the cancer cells, the camptothecin-derivative prodrug diffuses from the liposomes and/or micelles to be taken up by the cancer cells.

5        Any lipid or mixture of lipids which forms liposomes and/or micelles is suitable for use in the present invention. Phosphatidylcholines, including, for example, L-.alpha.-dimyristoylphosphatidylcholine (DMPC), L-.alpha.-dipalmitoylphosphatidylcholine (DPPC) and L-.alpha.-distearoylphosphatidylcholine (DSPC) are suitable. Also, phosphatidylglycerols, including, for example, L-.alpha.-dimyristoylphosphatidylglycerol (DMPG) are suitable. The DMPC and DMPG are both fluid phase at 37°C, while DSPC is solid phase at 37°C. Since the

10       presence of negatively charged lipid in the liposome membrane causes the liposomes to repel each other, small amounts, such as, for example about 10%, of an negatively charged lipid, such as distearolphosphotidylglycerol (DSPG), may be incorporated into the DSPC liposomes. Other suitable phospholipids include: phosphatidyl-ethanolamines, phosphatidylinositols, and phosphatidic acids containing lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic,

15       arachidonic, behenic and lignoceric acid. Another suitable lipid includes cholesterol.

The liposomes and/or micelles may be coated with polyethyleneglycol or $GM_1$ protein which assists the particles in avoiding the reticuloendothelial system.

DSPC, since it is solid phase at 37°C, (the average temperature of humans), limits the diffusion of the camptothecin drug from the liposome and thus can be employed to time release

20       the camptothecin-derivative prodrugs.

The DMPG, DPPC, and DSPC could be obtained from Avanti Polar Lipids, Alabaster, Ala. and used without further purification. All other chemicals can be reagent grade and used without further purification.

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Any surfactant or mixtures thereof, which forms micelles is suitable for use in the present invention. Suitable surfactants include sodium dodecylsulfate (SDS) available from Kodak, Rochester, N.Y., octylphenolpolyoxyethylene glycol, available under the trade name "Triton X-100" from Aldrich Chemical Co., Milwaukee, Wis., and sorbitan mono-oleate,

5    available under the trade name "Polysorbate 80" and "Tween 80" from Sigma Chemical Co. Other suitable surfactants include, for example, deoxycholic acid sodium salt, cholic acid sodium salt, and polyoxyethylene-10-cetylether, available under the trade name "BRIJ-56"; these surfactants are available from Sigma Chemical Co.

In addition, micelles may be composed of lipid, such as phospholipid, and mixtures of

10   lipids. Also micelles may be composed of both lipid and surfactant.

Liposome suspensions can be prepared by the method of Burke and Tritton Biochemistry 24: 1768-1776 (1985), which is incorporated herein in its entirety by reference. The liposomes are preferably small unilamellar vesicles (SUV), rather than multilamellar vesicles (MLV). However, both SUVs and MLVs are within the scope of the invention. While

15   MLVs have the advantage of limiting the rate of diffusion of the associated camptothecin-derivative prodrug, they have the disadvantage of being more easily scavenged by macrophages than the SUVs. Stock lipid suspensions containing 200 mg/mL lipid in phosphate-buffered saline (PBS) containing 8 mM $Na_2HPO_4$, 1 mM $KH_2PO_4$, 137 mM NaCl, and 3 mM KCl having a pH of 7.4 can be used and are prepared by vortex mixing for 5-10 min above the gel-

20   liquid-crystalline phase transition temperature $T_M$ of the lipid. The lipid suspensions are then sonicated using a bath-type sonicator from Laboratory Supplies Co., Hicksville, N.Y., for 3-4 hours until they become optically clear. A decrease in pH from 7.4 to 6.8 may be observed for the SUV preparations of DMPG; therefore, the pH of these SUV suspensions is preferably

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adjusted to 7.4 using small quantities of 2.5M NaOH in PBS and sonicated again. Each type of

liposome suspension is preferably annealed for 30 minutes at 37°C.

The preparations of many liposomes and micelles are described in U.S. Patent Nos.

5,552,156, and 5,736,156, which are herein incorporated in their entireties by reference.

5          The liposomal prodrugs of the present invention can be made, for example, in the

following manner, beginning with the formation of the CPT-derivative compound. 20(S)-

camptothecin or 9-nitrocamptothecin can be reacted with organic anhydrides in pyridine for

example. In particular, the anhydride is mixed with pyridine in about a 1:1 ratio to provide

a homogeneous solution to which the 20(S)-camptothecin or 9-nitrocamptothecin is added all

10        at once. The mixture is stirred under atmospheric pressure for 24 to 48 hours. At the end of

the reaction time, the mixture is poured onto a certain amount of petroleum ether while

stirring. The product, precipitated from petroleum ether, is collected by filtration and washed

with petroleum ether several times. The purity of the product by this procedure is usually

98% (analyzed by HPLC). This procedure is applicable to all of the compounds of the present

15        invention except where $R_1$ is $C_7$-$C_{15}$ alkyl or alkenyl, or an epoxy group in Formula (I). The

derivative where $R_1$ is a $C_7$-$C_{15}$ alkyl is obtained by the reaction of camptothecin with

nonanoyl chloride in methylene chloride under reflux. The derivative where $R_1$ contains an

epoxy moiety is obtained by epoxidation of the compound of the present invention, where $R_1$

is an alkenyl group in Formula (I). The 9-aminocamptothecin derivatives of the present

20        invention can be made by taking the 9-nitrocamptothecin derivatives of the present invention

and reducing them, such as with iron powder under acidic conditions, to form the 9-

aminocamptothecin derivatives of the present invention.

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As a specific example, approximately 0.5 g of the 9-nitrocamptothecin derivatives can be mixed with about 100 mg of iron powder and 20 ml of 10% HCl to form the 9-aminocamptothecin derivative.

The CPT-derivative prodrug compound is then incorporated, encapsulated,
5   surrounded, entrapped, or otherwise restrained in, on, or by liposomes and/or micelles, for example, liposomes comprised of lipids such as cholesterol, or phospholipids, or micelles comprised of surfactants such as, for example, sodium dodecylsulfate, octylphenylpolyoxyethylene glycol, or sorbitan mono-oleate. Preferably, the CPT-derivative prodrug compound is solubilized in the liposomes and/or micelles.

10   The liposomal prodrugs of the present invention are effective in treating malignant tumors or cancer in a mammal. The compounds of the present invention can be administered in any fashion known to those skilled in the art. Preferably, the compounds are administered intramuscularly, orally, or transdermally.

As used herein, the term "malignant tumor" is intended to encompass all forms of
15   human carcinomas, sarcomas and melanomas which occur in the poorly differentiated, moderately differentiated, and well differentiated forms.

The compounds of the present invention and formulations of the present invention can be used in the treatment of a number of tumors and/or cancers including, but not limited to, human cancers of the lung, breast, colon, prostate, melanoma, pancreas, stomach, liver, brain,
20   kidney, uterus, cervix, ovaries, urinary track, gastrointestinal, and other solid tumors which grow in a anatomical site other than the blood stream as well as blood borne tumors such as leukemia. Other solid tumors include, but are not limited to, colon and rectal cancer. The compounds of the present invention are also useful as inhibitors of the enzyme topoisomerase I.

The compounds of the present invention can be administered by any acceptable route including, but not limited to, orally, intramuscularly, transdermally, intravenously, through an inhaler or other air borne delivery systems, and the like. Preferably, the compounds and the formulations of the present invention are administered orally, intramuscularly, or transdermally and most preferably delivered orally. Examples of transdermally delivery systems can be found, for instance in U.S. Patent No. 5,552,154 and 5,652,244 incorporated in their entirety by reference herein. The compounds or formulations of the present invention can also be administered to a patient through a liposome system such as ones described in U.S. Patent Nos. 5,882,679; 5,834,012; 5,783,211; 5,718,914; 5,631,237; 5,552,156; 5,059,421; 5,000,958; 5,874,105; 5,567,434; 5,549,910; 5,043,165; 5,736,156; 5,567,433; and 4,663,161, all incorporated in their entirety by reference herein.

In addition, the compounds and formulations of the present invention can be used in combination with other drugs and formulations for the treatment of cancers such as taxol, taxotere, or their derivatives as well as cisplatin and derivatives thereof.

In treating or retarding malignant tumors in mammals in accordance with the present invention, the aforedescribed liposomal prodrugs of the present invention are administered by means known to those skilled in the art, and are preferably administered intramuscularly, transdermally, or orally, using commonly known methods, for example, gelatin capsules for oral administration, as well as formulations such as microsuspensions of the liposomal prodrugs in lipid and in lipid-like emulsions (e.g. - Intralipid 20, cottonseed oil and peanut oil) for intramuscular administration and inclusion in cholesterol pellets for subcutaneous long-term administration.

As used herein, an "effective amount" of the liposomal CPT-derivative prodrugs of the present invention is intended to mean that amount of the compound which will inhibit the

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growth of, or retard cancer, or kill malignant cells, and cause the regression and palliation

of malignant tumors, i.e., reduce the volume or size of such tumors or eliminate the tumor

entirely.

With mammals, including humans, the effective amounts can be administered on the

5    basis of body surface area. The interrelationship of dosages varies for animals of various

sizes and species, and for humans (based on mg/M$^2$ of body surface) is described by E.J.

Freireich et al., Cancer Chemother. Rep., 50(4) :219 (1966). Body surface area may be

approximately determined from the height and weight of an individual (see, e.g., Scientific

Tables, Geigy Pharmaceuticals, Ardsley, N.Y. pp. 537-538 (1970). An effective amount of

10   the camptothecin compounds in the present invention can range from about 12.5 to about 31.3

mg/m$^2$ of body surface per day, and for the liposomal prodrugs an effective amount can range

from about 12.5 to about 3000 mg/m$^2$ of body surface area per day based on the weight of

the prodrug and the liposomal delivery system.

The preferred effective amounts or dosages of the liposomal prodrugs of the present

15   invention in mice are about 1 to about 400 mg lipsomal prodrug per kg of body weight twice

a week for an intramuscular route and about 0.75 to about 150 mg liposomal prodrug/kg/day

for the oral route. Effective amounts or dosages of the lipsomal prodrugs of the present

invention in mice are, for instance, about 1.5 mg/kg/week to about 1000 mg/kg/week of the

lipsomal prodrug for the transdermal route. For all of the administering routes, the exact

20   timing of administration of the dosages can be varied to achieve optimal results. Generally,

when using Intralipid 20 as the liposomal carrier for the CPT-derivative, the actual dosage

of CPT-derivative reaching the patient will be less. This is due to some loss of the CPT-

derivative on the walls of the syringes, needles and preparation vessels, which is prevalent

with the Intralipid 20 suspension. Generally, about 1 mg to about 4 mg of CPT-derivative is added to about 0.1 ml to about 1 ml of lipid carrier.

Another important feature of the method provided by the present invention relates to the relatively low or no apparent overall toxicity of the liposomal prodrugs administered in accordance with the teachings herein. Overall toxicity can be judged using various criteria. For example, loss of body weight in a subject over 10% of the initially recorded body weight (i.e., before treatment) can be considered as one sign of toxicity. In addition, loss of overall mobility and activity and signs of diarrhea or cystitis in a subject can also be interpreted as evidence of toxicity. In one of the examples which follow, the overall toxicity of the camptothecin compounds of the present invention was evaluated.

The compounds used in the present invention may first be combined with pharmaceutically acceptable carriers or diluents, such as Intralipid 10 or 20 or natural oils, or other suitable emulsifiers for lipophilic compounds, prior to being incorporated, encapsulated, surrounded, entrapped, or otherwise restrained in, on, or by the lipsomal delivery system.

Another method of administering the liposomal prodrugs of the present invention is by a transdermal or transcutaneous route. One example of such an embodiment is the use of a patch. In particular, a patch can be prepared with a fine suspension of a liposomal prodrug of the present application in, for example, dimethylsulfoxide (DMSO), or a mixture of DMSO with cottonseed oil and brought into contact with the skin of the tumor carrying mammals away from the tumor location site inside a skin pouch. Other mediums or mixtures thereof with other solvents and solid supports would work equally as well for delivering the liposomal prodrugs. The patch can contain the CPT-derivative-containing liposomal prodrug of the present invention in the form of a solution or a suspension. The patch can then be
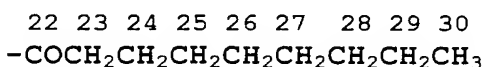
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applied to the skin of the patient, for example, by means of inserting it into a skin pouch of the patient formed by folding and holding the skin together by means of stitches, clips or other holding devices. This pouch should be employed in such a manner so that continuous contact with the skin is assured without the interference of the mammal. Besides using a skin

5    pouch, any device can be used which ensures the firm placement of the patch in contact with the skin. For instance, an adhesive bandage could be used to hold the patch in place on the skin.

The present invention will be further clarified by the following example, which is intended to be purely exemplary of the present invention.

10

## EXAMPLES

All glassware referenced in the examples was baked at 80-100°C for a minimum of 2 hours before being used. Melting points were obtained with a MEL-TEMP melting point apparatus and were uncorrected. The $^1$H and $^{13}$C NMR spectra were obtained at 270.05 MHZ

15    with a JEOL GX-270 WB NMR spectrometer. Chemical shifts are reported in parts per million ($\delta$ scale), employing tetramethylsilane as an internal standard. In reporting the NMR data, the following abbreviations are used: coupling constants in Hertz (J), singlet (s), doublet (d), triplet (t), broad singlet (brs), multiplet (m), and etc. Mass Spectra were recorded using a VG ZAB - SEQ mass spectrometer (VG Analytical Co., England) with a resolution of

20    10000. The numbering system used to depict NMR for camptothecin portion is shown in formula (X). The numbering for the side chain is shown as below:

$$\overset{22}{-}\overset{23}{CO}\overset{24}{CH_2}\overset{25}{CH_2}\overset{26}{CH_2}\overset{27}{CH_2}\overset{28}{CH_2}\overset{29}{CH_2}\overset{30}{CH_2}CH_3$$

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### EXAMPLE 1    -    Camptothecin 20 - O - propionate

In a 100 ml round - bottomed flask were mixed 25 ml propionic acid anhydride and

20 ml pyridine.  A homogeneous solution was obtained after shaking for 30 s.  To this

solution, 2.0 g of starting camptothecin was suspended.  The mixture was stirred at 40 $\pm$ 5$^0$C

5     for 48 h.  After cooling to room temperature, the reaction mixture was poured onto 400 ml

petroleum ether while stirring.  The product precipitated from petroleum ether was collected

by filtration and washed with 150 ml petroleum ether (50 ml x 3).  After drying under air for

1 h, a white powder, 2.17 g, was obtained.  The purity of the product which was measured

by HPLC was 98%.  Yield 94%, mp 250 - 252$^0$C (dec.).  $^1$HNMRin CDCl$_3$: 0.98 (3H, t, J

10    = 7.5 Hz, 19 - methyl protons), 1.17 (3H, t, J 7.51 Hz, 24 - methyl protons), 2.12 - 2.34

(2H, m, 18 - methylene protons), 2.48 -2.58 (2H, m, 23 - methylene protons), 5.29 (2H, s,

5 - methylene protons), 5.39 - 5.72 (2H, dd, J = 17.12, 17.12 Hz, 17 - methylene protons),

7.23 (1H, s, 14 - H), 7.68 (1H, t, J = 6.96 Hz, 10 -H), 7.84 (1H, t, J=6.96 Hz, 11 -H),

7.95 (1H, d, J=8.43 Hz, 9-H), 8.23 (1H, d, J=8.06Hz, 12-H), 8.40(1H, s, 7-H); $^1$H NMR

15    in TFA: 1.18(3H,t,J=7.5Hz, 19-methyl protons), 1.32 (3H, t, J = 7.30 Hz, 24 - methyl

protons), 2.30 - 2.80 (4H, m, 18 - and 23 - methylene groups), 5.60 - 6.10 (4H, s + dd, s

at 5.86 for 5 - methylene protons, dd with J = 18.96, 18.32 Hz for 17 -methylene protons),

7.99 (1H, s, 14 - H), 8.19 (1H, t, J = 8.06 Hz, 10 - H), 8.20 - 8.46 (2H, m, 9 - Hand 11

- H), 8.54 (1H, d, J = 8.79 Hz, 12 - H), 9.43 (IH, s, 7 - H); $^{13}$C NMR (TFA): 7.42 (C19),

20    8.55 (C24), 28.61 (C18), 33.07 (C23), 53.14 (C5), 68.77 (C17), 78.31 (C20), 105.68,

113.19, 117.35, 125.87, 131.23, 131.36, 132.59, 133.40, 139.30, 139.89, 140.78, 144.62,

147.00, 149.43 (C2, C3, C6 - C16, C16a), 172.50, 179.76 (C21, C22); mass m/e (relative

intensity): 405 [(M+H)+, 100%], 404 (M+, 15%), 331 [(M -CH$_3$CH$_2$COO), 17%], 317

[(M -C$_2$H$_5$COO - CH$_3$ + H), 10%], 303 [(M- C$_2$H$_5$COO -CO), 15%], 287 [(M- C$_2$H$_5$COO - CO$_2$), 9%], 261(9%).

<u>EXAMPLE 2</u>    -    <u>Camptothecin 20 - O - butyrate</u>

Using 20 ml butyric anhydride, 18 ml pyridine, and 1.61 g camptothecin, the reaction is carried out in the same manner as in Example 1 whereby 1.77 g of the title compound was obtained as a brownish powder, yield 92%, mp 225 - 227$^0$C (dec.). $^1$H NMR in CDCl$_3$: 0.98 (6H, t, J = 7.51 Hz, 19 - and 25 - methyl groups), 1.65 - 1.74 (2H, m, 24 - methylene protons), 2. 14 - 2.30 (2H, m, 18 - methylene protons), 2.44 - 2.51 (2H, m, 23 - methylene protons), 5.29 (2H, s, 5 - methylene protons), 5.38 - 5.71 (2H, dd, J = 17.59, 17.59 Hz, 17 - methylene protons), 7.23 (1H, s, 14 - H), 7.68 (1H, t, J = 8.06 Hz, 10 - H), 7.84 (1H, t, J = 7.96 Hz, 11 - H), 7.95 (1H, d,J=6.96 Hz, 9-H), 8.23 (1H, d, J=8.06 Hz, 12-H), 8.40 (1H, s, 7-H); $^1$H NMR in TFA: 0.75 - 1.15 (6H, m, 19 - and 25 - methyl groups), 1.70 - 1.80 (2H, m, 24 - methylene protons), 2.10 - 2.80 (4H, m, 18 - and 23 - methylene groups), 5.50 - 6.00 (4H, s + dd, s at 5.73 for 5 - methylene protons, dd for 17 - methylene protons), 7.86 (1H, s, 14 - H), 8.05 (1H, s, 10 -H), 8.30 (2H, brs, 9 - H and 11 - H), 8.40 (1H, s, 12 - H), 9.30 (1H, s, 7 - H); $^{13}$C NMR (TFA): 7.23 (C19), 13.20 (C25), 19.20 (C24), 32.91 (C18), 36.91 (C23), 52.96 (C5), 68.58 (C17), 78.00 (C20), 105.56, 113.40, 113.50, 117.00, 117.10, 131.00, 132.40, 133.16, 139.06, 139.15, 140.00, 144.36, 146.90, 149.40 (C2, C3, C6 - C16, C16a), 172.50, 178.00 (C12, C22); mass m/e (relative intensity): 419 [(M+H)+, 100%], 331 [(M - C$_3$H$_7$COO), 17%], 303 [(M- C$_3$H$_7$COO - CO), 13%], 287 [(M- C$_3$H$_7$COO - CO$_2$), 8%], 273 (2%), 261(3%).

<u>EXAMPLE 3</u>    -    <u>Camptothecin 20 - O - valerate</u>

Using 15 ml valeric anhydride, 14 ml pyridine, and 1.51 g starting camptothecin, the reaction was carried out in the same manner as in Example 1 whereby 1.68 g of the title

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compound was obtained as a gray - white powder, yield 90%, mp 265°C (dec.).  $^1$H NMR in CDCl$_3$: 0.92 (3H, t, J = 7.33 Hz, 26 - methyl protons), 0.98 (3H, t, J = 7.51, 19 - methyl protons), 1.37 - 2.00 (4H, m, 24 - and 25 - methylene protons), 2.10 - 2.28 (2H, m, 18 - methylene protons), 2.46 - 2.53 (2H, m, 23 - methylene protons), 5.30 (2H, s, 5 -

5     methylene protons), 5.38 -5.71 (2H, dd, J = 17.22, 17.21 Hz, 17 - methylene protons), 7.23 (1H, s, 14 - H), 7.70 (1H, t, J = 6.96 Hz, 10 - H), 7.82 (1H, t, J = 6.96 Hz, 11 - H), 7.95 (1H, d, J = 7.32 Hz, 9 -H), 8.22 (1H, d, J= 8.42 Hz, 12 - H), 8.40 (1H, s, 7 - H);  $^1$H NMR In TFA: 0.83 (3H, brs, 26 - methyl protons), 0.99 (3H, brs, 19 - methyl protons), 1.32 (2H, m, 25 - methylene protons), 1.60 (2H, m, 24 -methylene protons), 2.19 - 2.58 (4H, m, 18

10    - and 23 - methylene protons), 5.49 - 5.82 (4H, s + dd, s at 5.67 for 5 - methylene protons, dd with J = 17.58, 18.68 Hz for 17 - methylene protons), 7.80 (1H, s, 14 - H), 7.99 (1H, s, 10 - H), 8.23 (2H, brs, 9 - H and 11 - H), 8.33 (1H, s, 12 -H), 9.24 (1H, s, 7 -H);  $^{13}$C NMR(TFA): 4.48 (C26), 10.37 (C19), 20.23 (C24), 24.98 (C25), 30.15 (C18), 32.03 (C23), 50.20 (C5), 65.82 (C17), 75.36 (C20), 102.84, 109.89, 110.24, 114.06, 114.39, 128.25,

15    128.39, 129.65, 130.41, 136.30, 137.00, 141.62, 148.57, 149.28 (C2, C3, C6 -C16, C16a), 169.00, 176.80 (C21, C22); mass m/e (relative intensity): 433 [(M+H)$^+$, 100%], 331, [(M-C$_4$H$_9$COO), 17%], 303 [(M- C$_4$H$_9$COO - CO), 13%], 287 [(M - C$_4$H$_9$COO - CO$_2$), 7%], 273 (2%), 261(4%).

<u>EXAMPLE 4</u>    -    <u>Camptothecin 20 - O - heptanoate</u>

20    Using 15 ml heptanoic anhydrides, 13 ml pyridine, and 1.55 g starting camptothecin, the reaction was carried out in the same manner as in Example 1 whereby 2.0 g of the title compound was obtained as a gray - white powder, yield 98%, mp 270°C (deformed at 210°C).  $^1$H NMR in CDCl$_3$: 0.82 (3H, t, J = 7.51 Hz, 28-methyl protons), 0.98 (3H, t, J = 7.01 Hz, 19 -methyl protons), 1.20 - 1.80 ( 8H, m, 24-, 25-, 26-, and 27 - methylene

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protons), 2.10 - 2.30 (2H, m, 18 - methylene protons), 2.40 - 2.60 (2H, m, 23 - methylene

protons), 5.29 (2H, s, 5 -methylene protons), 5.38 - 5.72 (2H, dd, J = 17.69, 17.22 Hz, 17

- methylene protons), 7.23 (1H, s, 14-H), 7.68 (1H, t, J=7.30 Hz, 10-H), 7.84 (1H, t, J =

7.42 Hz, 11-H), 7.95 (1H, d, J= 8.06 Hz, 9-H), 8.22 (1H, d, J=8.32Hz, 12-H), 8.40(1H,

5      s, 7-H); $^1$H NMR in TFA: 0.74 (3H, s, 28 - methyl protons), 0.99 (3H, s, 19 - methyl

protons), 1.21 (6H, brs, 25-, 26-, and 27 -methylene protons), 1.62 (2H, s, 24 - methylene

protons), 2.10 - 2.30 (4H, m, 18 - and 23 -methylene groups), 5.50 - 6.00 (4H, s + dd, s

at 5.67 for 5 - methylene protons, dd for 17 -methylene protons), 7.80 (1H, s, 14 - H), 7.99

(1H, s, 10 - H), 8.23 (2H, s, 9 - H and 11 - H), 9.24 (1H, s, 7 - H); $^{13}$C NMR(TFA): 8.63

10     (C19), 14.99 (C28), 24.66 (C27), 27.14 (C26), 31.07 (C24), 33.68 (C25), 34.29 (C18),

36.45 (C23), 54.34 (C5), 69.98 (C17), 79.50 (C20), 106.97, 114.39, 118.55, 127.11,

132.41, 133.79, 134.55, 140.46, 141.11, 142.00, 145.79, 148.14, 150.62, 153.00 (C2, C3,

C6 - C16, C16a), 180.57, 193.10 (C21, C22); mass m/e (relative intensity): 461 [(M+H)$^+$,

100%], 331 [(M- C$_6$H$_{13}$COO), 20%], 317 [(M - C$_6$H$_{13}$COO - CH$_3$ + H), 10%], 303 [(M-

15     C$_6$H$_{13}$COO - CO), 15%], 287 [(M - C$_6$H$_{13}$COO - CO$_2$), 8%], 273 (2%), 261 (2%).

<u>EXAMPLE 5</u>      -      <u>Camptothecin 20 - O - nonanoate</u>

To 40 ml methylene chloride in a 100 ml round - bottomed flask were added 5 ml

nonanoyl chloride and 2 g starting camptothecin. The mixture was stirred under reflux for

48 h. After the solvent was evaporated by a rotary evaporator, the residue was

20     chromatographically separated with methylene chloride - THF as eluent. The title compound,

169 mg, was obtained as a pale yellow powder, yield 6%, mp 180°C. $^1$H NMR in CDCl$_3$:

0.84 (3H, t, J = 6.60 Hz, 30 -methyl proyons), 1.02 (3H, t, J = 7.69 Hz, 19 - methyl

protons), 1.20 - 1.80 (12H, m, 24 - 29 methylene protons), 2.10 - 2.38 (2H, m, 18 -

methylene protons), 2.40 - 2.60 (2H, m, 23 -methylene protons), 5.33 (2H, s, 5 - methylene

protons), 5.40 - 5.80 (2H, dd, J = 17. 22, 17.22 Hz, 17 - methylene protons), 7.26 (1H, s,

14 - H), 7.71 (1H, t, J = 8.06 Hz, 10 - H), 7.88 (1H, t, J = 8.43Hz, 11 -H), 7.99 (1H, d,

J=7.33 Hz, 9-H), 8.26 (1H, d, J = 8.79 Hz, 12-H), 8.44 (1H, s, 7 -H); $^1$HNMR in TFA:

0.96 (3H, s, 30 - methyl protons), 1.24 (3H, s, 19 - methyl protons), 1.38 (10H, brs, 25 -

29 - methylene protons), 1.87 (2H, m, 24 - methylene protons), 2.40 - 2.90 (4H, m, 18 - and

23 - methylene protons), 5.74 - 6.07 (4H, s + dd, s at 5.91 for 5 - methylene protons, dd

with J = 17.90, 18.21 Hz for 17 - methylene protons), 8.05 (IH, s, 14 - 11), 8.24 (1H, t, 10

-H), 8.48 (2H, m, 9 -H and 11-11), 8.57(1H, d, 12-H), 9.48 (1H, s, 7-11); $^{13}$C NMR (TFA):

4.99 (C30), 11.45 (C19), 21.17 (C29), 23.53 (C28), 27.82 (C24, C26 - 27), 30.52 (C25),

30.63 (C18), 32.80 (C23), 103.28, 110.73, 114.91, 123.47, 128.79, 128.90, 130.14, 130.93,

136.84, 137.46, 138.33, 142.17, 144.47, 146.94 (C2, C3, C6 - C16, Cl6a), 169.98, 176.92

(C21, C22); mass m/e (relative intensity): 489 [(M+H)$^+$, 100%], 33 1[(M - C$_8$H$_{17}$COO,

23%], 317 [(MC$_8$H$_{17}$COO - CH$_3$ + 11), 13%], 303 [(M- C$_8$H$_{17}$COO - CO), 17%], 287 [(M-

C$_2$H$_{17}$COO - CO$_2$), 8%], 273 (3%), 216 (2%).

<u>EXAMPLE 6     -     Camptothecin 20 - O crotonate</u>

Crotonic anhydride (40 ml) and pyridine (30 ml ) were mixed in a 100 ml round -

bottomed flask. To this solution, the starting camptothecin (8 g) was added. The mixture was

stirred at 90 ± 10°C for 15 h. After cooling to room temperature, the crude product was

precipitated in 1000 ml petroleum ether and collected by filtration. After column

chromatography, the compound (3 g) was obtained, yield 31%, mp 218 - 220°C (deformed

at 155°C). $^1$H NMR(CDCl$_3$): 1.00 (3H, t, J= 7.51 Hz, 19 - methyl protons), 1.94 ( 3H, dd,

J$_{H25\,H24}$=6.96HZ, J$_{H25-H23}$= 1.89Hz, 25 -methyl protons), 2.15 - 2.35 (2H, m, 18 -methylene

protons), 5.28 (2H, s, 5 - methylene protons), 5.38 - 5.74 (2H, dd, J = 17.22, 17.22 Hz,

17 -methylene protons), 5.99 (1H, d, J = 13.92 Hz, 23 - H), 7.05 - 7.14 (1H, dq, J$_{H24-H23}$

= 15.38 Hz, $J_{H24-H25}$ = 6.96 Hz, 24 - H), 7.24 (1H, s, 14 - H), 7.67 (IH, t, J = 6.96 Hz,

10-H), 7.83 (1H, t, J = 6.96Hz, 11 -H), 7.94 (1H, d, J = 7.70 Hz, 9-H), 8.21 (IH, d, J =

8.43 Hz, 12-H), 8.39 (1H, s, 7 - H); mass m/e (relative intensity): 416 ($M^+$, 20%), 330 [(M

- $CH_3CH=CHCOOH$), 100%], 315 [(M- $CH_3CH=CHCOOH$ - $CH_3$), 40%], 302 [(M-

$CH_3CH=CHCOOH$ - CO), 73%], 287 [(M- $CH_3CH=CHCOOH$ - $CO_2$ + 11), 30%], 274

(10%), 259 (9%), 246 (9%), 234 (3%), 218 (5%), 205 (4%), 191(3%); precise mass: found

416.137, $C_{24}H_{20}N_2O_5$ requires 416.137.

<u>EXAMPLE 7</u>      -      <u>Camptothecin 20 - O - 2', 3' - epoxy - butyrate</u>

To 50 ml benzene in a 100 ml round - bottomed flask were added 160 mg of the

starting compound of Example 6 and  150 mg m - chloroperoxybenzoic acid (57 - 86 %,

Aldrich Chemical Co., Milwaukee, WI). The mixture was stirred at room temperature for

a week. The solvent was removed by a rotary evaporator. The residue was

chromatographically separated. The compound (120 mg) was obtained as white powder, yield

72%, mp: the compound deformed at $175^0$C and decomposed at 210 - $213^0$C. $^1$H NMR

(CDCl$_3$): 0.99 (3H, t, J= 7.51 Hz, 19 - methyl protons), .90 - 1.94 (3H, dd, $J_{H25-H24}$ =

6.96Hz, $J_{H25-H23}$ = 1.84Hz, 25-methyl protons), 2.07-2.33 (2H, m, 18 -methylene protons),

5.30 (2H, s, 5 - methylene protons), 5.38 - 5.72 (2H, dd, J = 17.59, 17.95 Hz, 17 -

methylene protons), 5.95 - 6.02 (IH, dd, $J_{H23-H24}$ = 15.75 Hz, $J_{H23-H25}$ = 1.83 Hz, 23 -H),

7.04 - 7.12 (1H, dq, $J_{H24-H25}$ = 6.59 Hz, $J_{H24-H23}$ = 15.38 Hz, 24 - H), 7.22 (1H, s, 14 - H),

7.75 - 8.01 (4H, m, 9 - 12 aromatic protons), 8.78 (1H, d, J = 8.06 Hz, 7 - H); mass m/e

(relative intensity): 432 ($M^+$, 28%), 416 [(M- O), 12%], 346 [(M- $C_4H_6O_2$), 100%], 331 [(M-

$C_4H_5O_3$), 53%], 318 [(M- $C_4H_6O_2$ - CO), 75%], 303 [(M- $C_4H_5O_3$ - CO), 54%], 287 [(M-

$C_4H_5O_3$ - $CO_2$), 27%], 275 (15%), 259 (7%), 246 (8%), 231 (5%), 218 (8%), 205 (10%),

191 (5%); precise mass: found 432.132, $C_{24}H_{20}N_2O_6$ requires 432.132.

## EXAMPLE 8    -    9 - Nitrocamptothecin 20 - O - acetate

Acetic anhydride (3 ml) and pyridine (2 ml) were mixed in a 50 ml round - bottomed

flask in which 140 mg 9 - nitrocamptothecin was placed. The mixture was stirred at room

temperature for 24 h. The mixture was then separated by chromatotron. The title compound,

70 mg, was obtained as a yellow powder, yield 45%, mp 195°C (deformed at 165°C). $^1$H

NMR (CDCl$_3$): 1.02 (3H, t, J = 7.51 Hz, 19 - methyl protons), 2.10 - 2.40 (5H, s + m, s

at 2.26 for 23 - methyl protons, m for 18 - methylene protons), 5.40 (2H, s, 5 - methylene

protons), 5.41 - 5.75 (2H, dd, J = 17.59, 17.95 Hz, 17 - methylene protons), 7.23 (1H, s,

14 - H), 7.96 (1H, t, J = 6.96 Hz, 11 -H), 8.53 (1H, d, J 10.99 Hz, 10 - H), 8.58 (1H, d,

J = 9.98 Hz, 12 - H), 9.31 (1H, s, 7 - H); mass m/e (relative intensity): 435 (M$^+$, 25%), 375

[(M- CH$_3$COOH), 100%], 360 [(MCH$_3$COOH - CH$_3$), 40%], 347 [(M- CH$_3$COOH - CO),

87%], 332 [(M- CH$_3$COOH - CO -CH$_3$), 37%], 319 (13%), 302 (11%), 291 (10%), 286

(11%), 274 (10%), 258 (4%), 246 (5%), 216 (8%); precise mass: found 435.107, C$_{22}$H$_{17}$N$_3$O$_7$

requires 435. 107.

## EXAMPLE 9    -    9- Nitrocamptothecin - 20 - O - propionate

Using 6 ml propionic anhydride, 5 ml pyridine, and 600 mg starting 9-

nitrocamptothecin, the reaction was carried out in the same manner as in Example 8. After

the reaction, the crude product was allowed to precipitate from 200 ml petroleum ether,

collected by filtration, separated by column chromatography, and purified by reprecipitation

from 200 ml petroleum ether. The pure compound (500 mg) was obtained as a yellow

powder, yield 73%, mp 163°C (deformed at 155°C). $^1$H NMR(CDCl$_3$): 0.99 (3H, t, J 7.51

Hz, 19 - methyl protons), 1.18 (3H, t, J= 7.46 Hz, 24 - methyl protons), 2.10 - 2.30 (2H,

m, 18- methylene protons), 2.52 - 2.70 (2H, m, 23 -methylene protons), 5.37 (2h, s, 5 -

methylene protons), 5.39 - 5.73 (2H, dd, J = 17.58, 17.58 Hz, 17 - methylene protons),

7.22 (1H, s, 14 - H), 7.93 (1H, t, J = 8.06 Hz, 11 - H), 8.50 (1H, d, J = 10.60 Hz, 10 -

H), 8.54 (1H, d, J = 8.43 Hz, 12 - H), 9.28 (1H, s, 7 - H); mass m/e (relative intensity):

449 (M$^+$, 28%), 375 [(M- $C_2H_5COOH$), 100%], 360 [(M - $C_2H_5COOH$ - $CH_3$), 35%], 347

[(M- $C_2H_5COOH$ - CO), 82%], 332 [(M - $C_2H_5COOH$ - CO - $CH_3$), 26%], 319 (9%), 302

5    (8%), 291(7%), 274 (7%), 258 (2%), 245 (2%), 216 (2%); precise mass: found 449.122,

$C_{23}H_{19}N_3O_7$ requires 449. 122.butyrate.

### EXAMPLE 10    -    9 - Nitrocamptothecin 20 - O - butyrate

Using 2 ml butyric anhydride, 2 ml pyridine, and 60 mg starting 9 -

nitrocamptothecin, the procedure for preparation of the compound was the same as Example

10    8. The title compound (40 mg) was obtained as a yellow powder, yield 56%, mp 182$^0$C. $^1$H

NMR (CDCl$_3$): 0.98 (6H, m, 19 - and 25 - methyl groups), 1.65 - 1.70 (2H, m, 24 -

methylene protons), 2.10 - 2.40 (2H, m, 18 -methylene protons), 2.41 - 2.60 (2H, m, 23 -

methylene protons), 5.36 (2H, s, 5 - methylene protons), 5.38 - 5.72 (2H, dd, J = 17.59,

17. 96 Hz, 17 - methylene protons), 7.22 (1H, s, 14 -H), 7.92 (1H, t, J = 7.52 Hz, 11 - H),

15    8.49 (1H, d, J = 10.80 Hz, 10 - H), 8.53 (1H, d, J = 9.53 Hz, 12 - H), 9.27 (1H, s, 7 - H);

mass m/e (relative intensity): 463 (M+, 14%), 375 [(MC$_3$H$_7$COOH), 100%], 360 [(M-

C$_3$H$_7$COOH - CH$_3$), 32%], 347 [(M - C$_3$H$_7$COOH - CO), 78%], 332 [(M- C$_3$H$_7$COOH - CO

- CH$_3$), 25%], 319 (9%), 302 (8%), 291(7%), 274 (7%), 258 (2%), 245 (3%), 216 (5%);

precise mass: found 463.137, $C_{24}H_{21}LN_3O_7$ ..

20                                            ### EXAMPLE 11

Identical cultures of HL-60 cells received 0.2 μM of 20(S) CPT or 0.2 μM of a CPT

ester (identified in Figure 17) and cell cycle perturbations and apoptosis were detected by

flow cytometry analysis of the relative DNA count using an Epics-Elite laser flow cytometry

(Coulter) and analyzed with the Multicycle program (Phoenix Flow Systems). Histograms

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A,H,Q: untreated cells; B,I,P: + CPT; C,J,Q: + CPT-acetate; D,K,R:+SF6A; E,L,S: +

SF6B; F,M,T: + SF6C; G,N,U: + SF6D. The cells were treated for 24 hr

(A,B,C,D,E,F,G), 72 hr (H,I,J,K,L,M,N) and 120 hr (O,P,Q,R,S,T,U). See Figure 17 for

meaning of SF6 numbers. $G_1$ = G0 + G1 cells; G2 = G2 + M cells; AP = apoptotic cells.

The number in parentheses indicates percent of apoptotic cells in the cell culture. The results

are shown in Figure 18.

## EXAMPLE 12

U-937 cells were treated with 0.2 µM of each compound identified in Figure 17

(including 20(S) CPT), then analyzed by flow cytometry as reflected in Fig. 18. U-937 cells

were untreated (AA,FF,KK) or treated with 0.2µM of CPT-acetate (BB,GG,LL), SF6C

(CC,HH,MM) SF6A (DD,II,NN) and SF6B (EE,JJ,OO) for 6 days (BB,CC,DD,EE), 8 days

(FF,GG,HH,II,JJ) and 10 days (KK,LL,MM,NN,OO), then subjected to flow cytometry

analysis. The results are set forth in Figures 19a and 19b.

## EXAMPLE 13

Human breast tumors were grown as xenografts in nude mice and were allowed to

reach measurable size before treatment with SF6A was initiated. Tumor bearing but

untreated mice served as a control (A,F). SF6A was administered intragastrically using doses

of 5 mg/kg (B,G), 6 mg/kg (C,H), 7 mg/kg (D,I) and 8 mg/kg (E,J). Maintenance,

xenografting, drug treatment and tumor measurements were performed according to

procedures described above. Tumor size (in cm³) and body weight (in g) were measured as

functions of the treatment period (in days). Tumor size: panels A,B,C,D,E,; and Body

weight: panels F,G,H,I,J are shown in Figure 20.

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EXAMPLE 14

Growth inhibition of U-937 cells treated with various CPT esters.

Each identified compound was added to reach a culture of U-937 cell having a final

concentration of 0.2 µM. The treatment time was for 120 hr. Growth inhibition in each of

5    the treated cultures was then estimated as a percent of the exponential growth of the cells that

was considered 100%. Each estimate was the mean value of four estimates. The results are

set forth on the Table below.

| Treatment | Growth % |
|-----------|----------|
| Untreated cells | 100 ± 2 |
| +CPT | 0.0 |
| +CPT-acetate | 92 ± 4 |
| +SF6A | 31 ± 2 |
| +SF6B | 44 ± 2 |
| +SF6C | 96 ± 5 |
| +SF6D | 92 ± 5 |
| +SF6E | 94 ± 4 |

EXAMPLE 15

Determination of SF6A (Prodrug 2) and CPT in liver homogenate.

20    Mouse liver was homogenized in 4 volumes of ice-cold 0.15 M KCl with the aid of

a Dounce homogenizer, then centrifuged at 15000 G for 10 min to remove particulate

material. The supernatant, designated as liver homogenate, was transferred into a capped

polypropylene tube, mixed with 2 µg SF6A/ml and incubated in a 37°C water-bath. At the

desired times, samples were removed and mixed with 4 volumes of ice-cold ethanol to

25    precipitate proteins. The samples were stored at -20°C until all were collected. Following

centrifugation at 15000 G for 10 min, the clarified supernatants were subjected to analysis

for determination of SF6A and CPT lactones by high pressure liquid chromatography

(HPLC). The compounds were detected by fluorescence spectroscopy setting the excitation and emission wavelengths at 347 nm and 418 nm, respectively. CPT and SF6A have characteristic retention times of 3.5 min and 13.5 min, respectively. The results are shown in the Table below.

| Incubation | Amount determined (μg/ml) | |
|---|---|---|
| time | SF6A | CPT |
| 0 min | 1.95 | 0.00 |
| 10 min | 2.06 | 0.00 |
| 60 min | 2.05 | 0.00 |
| 6 hr | 2.05 | 0.00 |
| 24 hr | 0.96 | 0.47 |
| 48 hr | 0.90 | 0.30 |

## EXAMPLE 16

Stability of lactone forms of CPT and SF6A in the human plasma.

CPT or SF6A lactone was added to human plasma (1 μg/ml) and an incubation and lactone determination were made as described in Example 15. The lactone amount at each time-point was detected after removing the carboxylate form, and subsequently was calculated as percent of the initial lactone amount added. The results shown in this Table below were derived from one of the three independent experiments performed using different incubation times. The pattern of quantitation of the lactones was similar in all three experiments.

| Incubation time (hr) | Percent in plasma | |
|---|---|---|
| | CPT lactone | SF6A lactone |
| 0.0 | 100.0 | 100.0 |
| 0.5 | 40.0 | NT |
| 1.0 | 12.0 | 86.0 |
| 2.0 | 0.5 | 77.0 |
| 4.0 | 0.0 | 68.0 |
| 6.0 | 0.0 | 56.0 |
| 27.0 | 0.0 | 9.0 |

## EXAMPLE 17

Swiss nude mice of the NIH high fertility strain were bred and maintained pathogen-free in a laboratory. (Giovanella, B. C. and Stehlin, J.S. "Heterotransplantation of human malignant tumors in 'nude' thymusless mice. I. Breeding and maintenance of 'nude' mice." *J. Natl. Cancer Inst.* 51: 615-619; 1973.)

Human malignant carcinomas of the colon, breast, lung, ovary, stomach, pancreas, bladder, prostate, osteosarcoma and malignant melanomas were heterotransplanted directly from patients into the nude mice and passaged serially. In particular, the tumors CLO, MUR, CAS, SW 48, SQU and BRO as described in Giovanella, B. C., Stehlin, J. S., Shepard, R.C. and Williams, L. J. "Correlation between response to chemotherapy of human tumors in patients and in nude mice." *Cancer* 52: 1146-1152; 1983, tumor SCH as described in Heim, S., Mandahl, N., Arheden, K., Giovanella, B.C., Yim, S. O., Stehlin, J. S., Jr. and Mitehman, F. "Multiple karyotypic abnormalities including structural rearrangements of 11P in cell lines from malignant melanomas." *Cancer Genet. Cytogent*, 35:5-20; 1988, and Verschraegen, C., Giovanella, B. C., Mendoza, J.T., Kozielski, A. J. and Stehlin, J. S., Jr. "Specific organ metastases of human melanoma cells injected into the arterial circulation of nude mice." *Anticancer Res., In Press*, and tumor PC-3 as described in *Invest. Urol.* 17, 16-

23, 1979, were used. Tumors DOY and HAR (undifferentiated non-oat cell carcinomas of the lung), DIL (a poorly differentiated squamous cell carcinoma of the lung), SPA (a moderately differentiated adenocarcinoma of the lung), LAN (an undifferentiated carcinoma of the ovary), and BRE (a moderately differentiated adenocarcinoma of the stomach) where heterotransplanted. For the experiments, the tumor tissue was finely minced in complete MEM medium and 0.5 ml of a 10%v/v suspension was inoculated subcutaneously on the upper back of groups of 10-30 mice. When the tumors became palpable and measurable in all the animals, they were divided into groups of 4-8 and treated with the desired dose of the drug in experiment or with the vehicle only for the controls.

A typical sample preparation for IM-injection or oral administration using Intralipid 20 or cottonseed oil for example includes the dispersion of the test compound by sonication (3 pulses for 30 seconds each) in Intralipid 20 or cottonseed oil at the standard concentration of 1 mg/ml using an Ultrasonic Processor, Model GE600 by Sonics and Materials, Inc., Conn. Intramuscular injections were performed through a 27-gauge needle into the deep muscles of the prosterior legs of the mice twice a week. The animals received up to 70 such injections consecutively without suffering ill effects except for some local fibrosis. Intravenous injections, using intralipid 20 as the suspending agent, were performed through a 30-gauge needle in one of the trial veins. No pulmonary embolism was observed after over 200 injections.

Oral administration was achieved by mixing the required amounts of drugs Intralipid 20 or cottonseed oil with a previously autoclaved dietary supplement composed of whole wheat bread saturated with whole milk. The supplement containing the drug was then thoroughly mixed to form a paste. Mice were fed 1 g of dietary supplement containing the required dose of the drugs once a day and then 5 g of autoclaved mouse feed. (At this

regime, the animals initially lose about 4g out of 30 g of body weight, regain 2 g within a few days, and stabilize at this level). This regime can be carried on indefinitely. Actually, the mice fed in this manner (a slightly lower maximum caloric intake) were healthier and longer-lived then mice fed ad lib (Giovanella, B. C., Shepard, R. C.,Stehlin, J.S., Venditti, J. M. and Abbott, B. J. "Calorie restriction: Effect on growth of human tumors heterotransplanted in nude mice." *J. Natl. Cancer Inst.* 68: 249-257; 1982). Oral administration was accomplished by injecting the mixture of compound with cottonseed oil directly into the stomach (IS). Table 1 summarizes some of the results obtained by the treatment with CPT and derivatives thereof.

TABLE 1

| Tumor | Treatment | # of Animals | Results | # of Treatments | Observations |
|---|---|---|---|---|---|
| Breast Ca CLO | IM, 2x/wk 4 mg/kg | 5 | 3/5 CR 2/5 PR | 13 | Day 63, 5/5 Alive, 4/5 Controls Alive |
|  |  |  |  |  |  |
| Breast Ca CLO | IM, 2x/wk 5 mg/kg | 5 | 3/5 PR 2/5 PR | 9 | Day 56, 4/5 Alive, 1 inj. Death, 5/5 Controls Alive |
|  | IM, 2x/wk 6 mg/kg | 5 | 2/5 PR 3/5 PR | 9 | 4/5 Alive, 1 inj. Death |
|  | IM, 2x/wk 7 mg/kg | 5 | 4/5 CR 1/5 PR | 9 | 5/5 Alive |
|  | IM, 2x/wk 8 mg/kg | 5 | 5/5 CR | 9 | 5/5 Alive |
|  |  |  |  |  |  |
| CLO | IM, 2x/wk 12 mg/kg | 3 | 3/3 CR | 26 | Day 110, 3/3 Alive, 0/4 Controls Alive |
|  | IM, 2x/wk 16 mg/kg | 4 | 4/4 CR | 26 | 3/4 Alive |
|  | IM, 2x/wk 20 mg/kg | 2 | 2/2 CR | 26 | 2/2 Alive |
|  |  |  |  |  |  |
| Colon Ca SQU | IS. 5+/2- 4 mg/kg | 6 | NR | 37 | Day 69, 0/6 Alive, 0/6 Controls Alive |
|  | IS. 5+/2- 8 mg/kg | 6 | 1 PR 5/6 NR | 37 | 0/6 Alive |

| | IS. 5+/2- 12 mg/kg | 6 | NR | 37 | 0/6 Alive |
|---|---|---|---|---|---|
| SW48 | IS. 5+/2- 20 mg/kg | 6 | 6/6 PR | 28 | Day 62, 6/6 Alive, 2/6 Controls Alive |
| SQU | IS. 5+/2- 30 mg/kg | 6 | 3/6 CR 2/6 PR 1/6 NR | 68 | Day 111, 5/6 Alive, 1/6 Controls Alive |
| | IS. 5+/2- 50 mg/kg | 6 | 5/6 CR 1 PR | 68 | 4/6 Alive |
| | IS. 5+/2- 70 mg/kg | 6 | 4/6 CR 2/6 PR | 68 | 5/6 Alive |
| | | | | | |
| Lung CA SPA | IS. 5+/2- 100 mg/kg | 4 | 3/4 CR 1/4 PR | 65 | Day 41, 4/4 Alive, 1/4 Controls Alive, Day 90 4/4 Alive, 1/4 Controls Alive, Day 107, 1/4 Alive, 1/4 Controls Alive |
| | | | | | |
| Colon CA HT29 | IS. 5+/2- 100 mg/kg | 5 | 1/5 CR 1/5 PR 3/5 NR | 48 (injection stopped, day 82) | Day 74, 4/5 Alive, 1/5 Controls Alive, Day 116 1/5 Alive, 0/5 Controls |
| | IS. 5+/2- 200 mg/kg | 5 | 3/5 CR 1/5 PR 1/5 NR | 48 (injection stopped, day 82) | Day 74, 4/5 Alive Day 116 2/5 Alive |
| | IS. 5+/2- 300 mg/kg | 5 | 4/5 CR 1/5 PR | 51 (injection stopped, day 87) | Day 74, 4/5 Alive Day 116 4/5 Alive |

[1]5 treated once a week
[2]7 treated once a week
[3]Tumor growth inhibitied
CR - Complete remission defined as total disappearance of the initial tumor, maintained for a least 30 days at teh making of this table
NR - No response
PR - Partial response
CA - Camptothecin
9-AC - 9-Nitro Camptothecin
9-NO₂CA - 9-Nitro Camptothecin
W - Week
D - Daily
Unless otherwise noted, all i.m. and i.v. doses are administered 2x week, all oral doses daily.

Figure 9 shows the effectiveness of a compound of formula (I) wherein $R_2$ is $NO_2$ and $R_1$ is a ethyl group (hereinafter "Compound IA"), against colon cancer when administered by interstomach means. One group of six nude mice where treated interstomach with 10 or 20 mg/kg body weight Compound IA in cottonseed oil per day for five consecutive days with two day intervals of no treatment (i.e., five days of treatment then two days no treatment, and five day treatment and so on). Another group of six nude mice were treated with 10 or 20 mg/kg body weight Compound IA in the same manner. There were also two control groups of six nude mice each. The designation of "fast" means no food was fed to the mice for at least 12 hours before treatment and the designation of "non-fast" means fed normally. Thus,

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in this experiment, there were 4 groups of mice with 6 mice in each group. The treatment was ended after 34 days and Figure 9 shows the results from day 13-34 wherein those mice receiving Compound IA saw a decline in the tumor size while the tumor size in the two control groups continued to increase in size.

5          Figure 10 shows the effectiveness of Compound IA on melanoma where the treatment regimen and number of mice were the same as in Figure 9 except there was only one control group and one Compound IA tested group. The treatment was ended after 20 days and as can be seen in Figure 10, the tumor size in the mice receiving Compound IA decreased in size while the tumor size in the control group increase dramatically.

10          Figure 11 shows the effectiveness of Compound IA on stomach cancer. The dosage regimen and manner of administration are the same as in Figure 9 except there were 5 mice per group and only one control group. Again, the Compound IA showed a prevention of the growth in the tumor size over a number of days until the experiment was terminated on day 34. The control group showed a dramatic increase in tumor size and only 2 of the 5 mice

15     survived the 84 day test.

          Figure 12 shows the effectiveness of Compound IA on lung cancer where the same dosage and administration regimen were used as in Figure 9 except there were 5 mice per group and only one control group. As can be seen from Figure 12, the tumor size showed a dramatic reduction over the length of the experiment which ended on day 34. None of the

20     mice in the control group survived the experiment and in fact all mice were dead by day 27.

          Figure 13 shows the effectiveness of Compound IA at high dosages against ovarian cancer. Unlike some other cancers, ovarian cancer was more resistant and thus high concentrations of Compound IA were more effective. The route of administration and the dosage regimen were the same as in Figure 9 except there were only 5 mice in each group

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and only one control group was used. The experiment ended after day 41 and all mice survived the experiment in each group. The Compound IA administered in higher dosage amounts was quite effective in reducing the tumor size while the control group and the group receiving the lesser dosage amount of Compound IA showed a steady increase in tumor size

5   throughout the experiment.

Figure 14 shows the effectiveness of Compound IA against human colon cancer. One group of 5 mice received Compound IA by intrastomach means as in Figure 9. The other group of 5 mice received the Compound IA in the same dosage amount by intramuscular means twice a week in the manner described above. As shown in Figure 14, the tumor size

10  in the groups of mice receiving the Compound IA increased slightly but then stabilized where there was no increase in the tumor size from day 11 to the end of the experiment which was on day 25. The control, on the other hand, showed a steady increase in tumor size throughout the experiment from day 6 to day 25.

Figure 15 shows the effectiveness of Compound IA against another form of human

15  colon cancer where there was one control group and one group of 6 mice that received Compound IA intramuscularly and one group of 6 mice that received the Compound IA by intrastomach means in the same manner as in Figure 14. As shown in Figure 15, the tumor size of the mice in the groups receiving Compound IA was essentially stabilized throughout the experiment which ended on day 111 while the tumor size for the control group showed

20  a large increase in size until the mice in the control group all died which was on day 53.

Figure 16 shows the effectiveness of Compound IB, which is a compound of formula (I) where $R_2$ is hydrogen and $R_1$ is a $C_3$ alkenyl group. Figure 16 also shows the effectiveness of Compound IB which is a group of formula (I) where $R_2$ is hydrogen $R_1$ is a $C_3$ epoxy ring.

In this experiment, a group of six mice received Compound IB in an amount of 8 mg/kg

body weight twice a week and another group of six mice received Compound IC, which is a compound of formula (I), where $R_2$ is hydrogen and $R_1$ is a $C_3$ epoxy ring, in the same amount twice a week. In each case, the mice received the drug by intramuscular means in the manner described above. The experiment was ended after day 55. Figure 16 shows the effectiveness of Compound IB in actually reducing the tumor size of the breast cancer, while Compound IC showed the ability to stabilize the tumor size after a certain amount of time and showed a lower tumor size than the control.

It is clear from these studies, that the CPT-derivatives of the liposomal prodrugs of the present invention have been demonstrated to possess an astonishing level of anticancer activity. This applies both to the spectrum of tumors covered and to the quality of the responses. The method of the present invention has been able to block growth completely and to totally regress human xenografts of carcinomas (e.g., lung, breast, colon, stomach, pancreas, bladder, prostate, osteosarcoma and ovaries) and malignant melanomas. This has been accomplished without any observable toxicity. Many of the mammals which have been treated continuously for six months have shown no ill effects nor regrowth of the tumor they once carried. Delivering these surprisingly effective CPT-derivatives in a long lasting liposomal form according to the present invention provides an anticancer treatment of extended release and prolonged activity.

Other embodiments of the present invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

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## WHAT IS CLAIMED IS:

1.      A liposomal prodrug comprising an active component and a liposomal delivery system for delivering said active component, said delivery system comprising a liposome, a micelle and/or a lipid membrane receptacle, which restrains said active component, said active component comprising a compound of the formula:
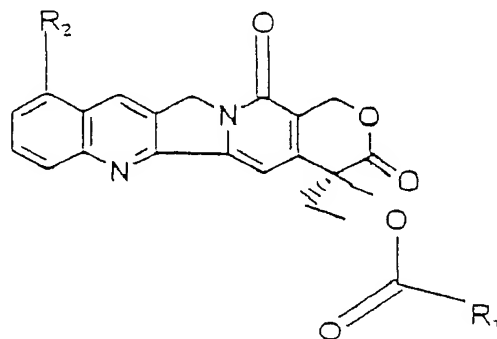


wherein $R_1$ is a $C_2$-$C_4$ alkyl group, a $C_6$-$C_{15}$ alkyl group, a $C_3$-$C_8$ cycloalkyl group, a $C_2$-$C_{15}$ alkenyl group or a $C_2$-$C_{15}$ epoxy group when $R_2$ is H; and $R_1$ is a $C_1$-$C_{15}$ alkyl group, a $C_3$-$C_8$ cycloalkyl group, a $C_2$-$C_{15}$ alkenyl group or a $C_2$-$C_{15}$ epoxy group when $R_2$ is $NH_2$ or $NO_2$.

2.      The liposomal prodrug of claim 1, wherein $R_1$ is a linear $C_2$-$C_4$ alkyl group or $C_6$-$C_{15}$ alkyl group when $R_2$ is H and $R_1$ is a linear $C_1$-$C_{15}$ alkyl group when $R_2$ is $NH_2$ or $NO_2$.

3.      The liposomal prodrug of claim 1, wherein $R_1$ is a linear $C_2$-$C_{15}$ alkenyl group or a $C_2$-$C_{15}$ epoxy group.

4.      The liposomal prodrug of claim 1, wherein $R_2$ is $NO_2$.

5.      The liposomal prodrug of claim 1, wherein $R_2$ is $NH_2$.

6.      The liposomal prodrug of claim 1, wherein $R_2$ is H.

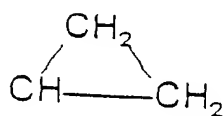7.    The liposomal prodrug of claim 2, wherein $R_1$ is a linear methyl, ethyl or propyl group and $R_2$ is $NO_2$.

8.    The liposomal prodrug of claim 1, wherein $R_1$ is a $C_3$-$C_8$ cycloalkyl group and $R_2$ is $NO_2$.
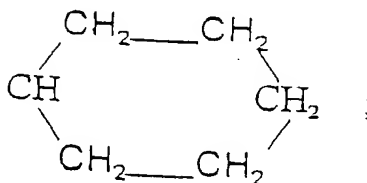
9.    The liposomal prodrug of claim 1, wherein $R_2$ is $NO_2$, and $R_1$ is $CH_3$; $CH_2CH_3$; or $CH_2CH_2CH_3$; $CH=CH_3$; $CH=CHCH_3$(trans) or
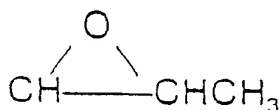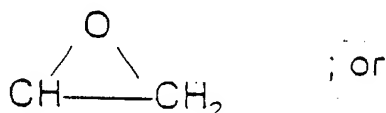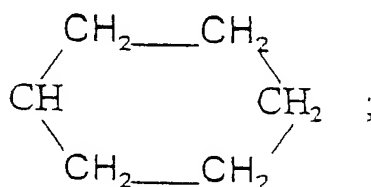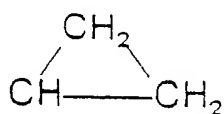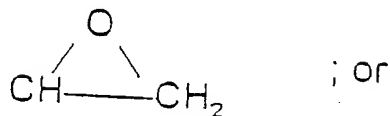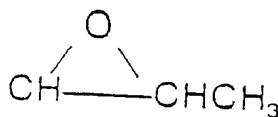
$$CH \underset{\displaystyle CH_2}{\overset{\displaystyle CH_2}{\bigtriangleup}} CH_2 \quad ;$$

$$CH \overset{\displaystyle CH_2—CH_2}{\underset{\displaystyle CH_2—CH_2}{\diagup\diagdown}} CH_2 \quad ;$$

$$CH \underset{\displaystyle CH_2}{\overset{\displaystyle O}{\bigtriangleup}} ; \text{ or}$$

$$CH \underset{\displaystyle CHCH_3}{\overset{\displaystyle O}{\bigtriangleup}}$$

10.    The liposomal prodrug of claim 1, wherein said delivery system comprises at least one of cholesterol and phospolipids.

11.    The liposomal prodrug of claim 1, wherein said delivery system comprises at least one unilamellar liposome.

12.    The liposomal prodrug of claim 1, wherein said delivery system comprises at least one micelle and said micelle comprises a surfactant.

13.    A method for treating cancer in a patient comprising administering a composition comprising an effective amount of the liposomal prodrug of claim 1, wherein said cancer is responsive to said composition.

14.    The method of claim 13, wherein $R_1$ is a linear $C_2$-$C_4$ alkyl group or a $C_6$-$C_{15}$ alkyl group where $R_2$ is H and $R_1$ is a linear $C_1$-$C_{15}$ alkyl group when $R_2$ is $NH_2$ or $NO_2$.

15.    The method of claim 13, wherein $R_1$ is a linear $C_2$-$C_{15}$ alkenyl group or a $C_2$-$C_{15}$ epoxy group.

16.    The method of claim 13, wherein $R_2$ is $NO_2$.

17.    The method of claim 13, wherein $R_2$ is H.

18.    The method of claim 14, wherein $R_1$ is a linear methyl, ethyl or propyl group and $R_2$ is $NO_2$.

19.     The method of claim 13, wherein $R_1$ is a $C_3$-$C_8$ cycloalkyl group and $R_2$ is NO$_2$.

20.     The method of claim 13, wherein $R_2$ is NO$_2$, and $R_1$ is CH$_3$; CH$_2$CH$_3$; or CH$_2$CH$_2$H$_3$; CH=CH$_3$; CH=CHCH$_3$(trans) or

5

10

$$\underset{\text{CH}\rule{1cm}{0.4pt}\text{CH}_2}{\overset{\text{CH}_2}{}} \quad ;$$

15

$$\underset{\underset{\text{CH}_2\rule{0.7cm}{0.4pt}\text{CH}_2}{\text{CH}}}{\overset{\text{CH}_2\rule{0.7cm}{0.4pt}\text{CH}_2}{}}\underset{\text{CH}_2}{} \quad ;$$

20

$$\underset{\text{CH}\rule{1cm}{0.4pt}\text{CH}_2}{\overset{\text{O}}{}} \quad ; \text{ or}$$

25

$$\underset{\text{CH}\rule{1cm}{0.4pt}\text{CHCH}_3}{\overset{\text{O}}{}} \quad .$$

21. The method of claim 13, wherein said delivery system comprises at least one of cholesterol and phospolipids.

22. The method of claim 13, wherein said delivery system comprises at least one unilamellar liposome.

23. The method of claim 13, wherein said delivery system comprises at least one micelle and said micelle comprises a surfactant.

24. A compound of formula (I):

(I)
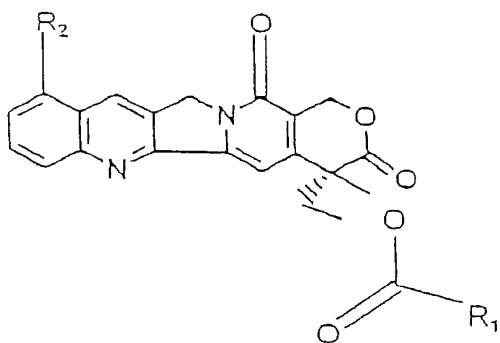


wherein $R_1$ is a $C_1$-$C_{15}$ alkyl group, a $C_3$-$C_8$ cycloalkyl group, a $C_2$-$C_{15}$ alkenyl group, or $C_2$-$C_{15}$ epoxy group and $R_2$ is $NH_2$.

25. The compound of claim 24, wherein $R_1$ is a linear $C_1$-$C_{15}$ alkyl group.

26. The compound of claim 24, wherein $R_1$ is a linear $C_2$-$C_{15}$ alkenyl group.

27. The compound of claim 24, wherein $R_1$ is $C_2$-$C_{15}$ epoxy group.

28. The compound of claim 24, wherein $R_1$ is a $C_3$-$C_8$ cycloalkyl group.

29. The compound of claim 24, wherein $R_1$ is a linear methyl, ethyl, propyl, butyl, hexyl or octyl group.

30. The compound of claim 24, wherein $R_1$ is ethyl.

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31.    A method for treating cancer in a patient comprising administering a composition comprising an effective amount of the compound of claim 24.

32.    The method of claim 31, wherein $R_1$ is a linear $C_1$-$C_{15}$ alkyl group.

33.    The method of claim 31, wherein $R_1$ is a linear $C_2$-$C_{15}$ alkenyl group.

34.    The method of claim 31, wherein $R_1$ is $C_2$-$C_{15}$ epoxy group.

35.    The method of claim 31, wherein $R_1$ is a linear ethyl, propyl, butyl, hexyl, or octyl group.

36.    The method of claim 31, wherein $R_1$ is ethyl.

37.    The method of claim 31, wherein $R_1$ is a linear methyl group.

38.    The method of claim 31, wherein $R_1$ is a $C_3$-$C_8$ cycloalkyl group.

Mouse CPT Plasma Levels

2 MG/KG, I.S.
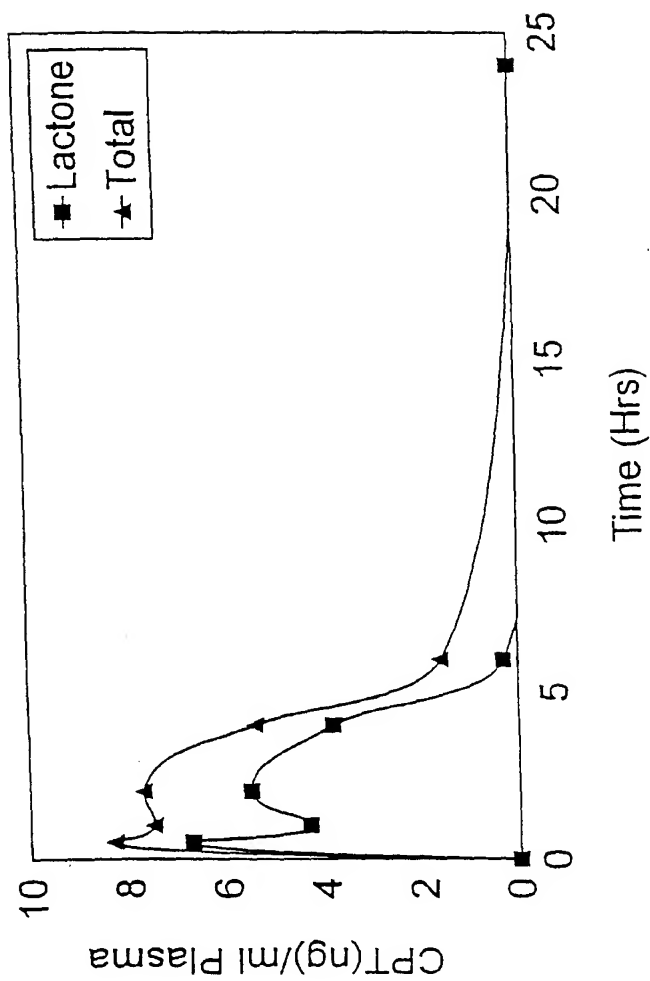
FIG. 1

Human CPT Plasma Levels

4 MG, P.O.

FIG. 2

Mouse 9 - NC Plasma Levels Day 1

1 Mg/kg, I.S.
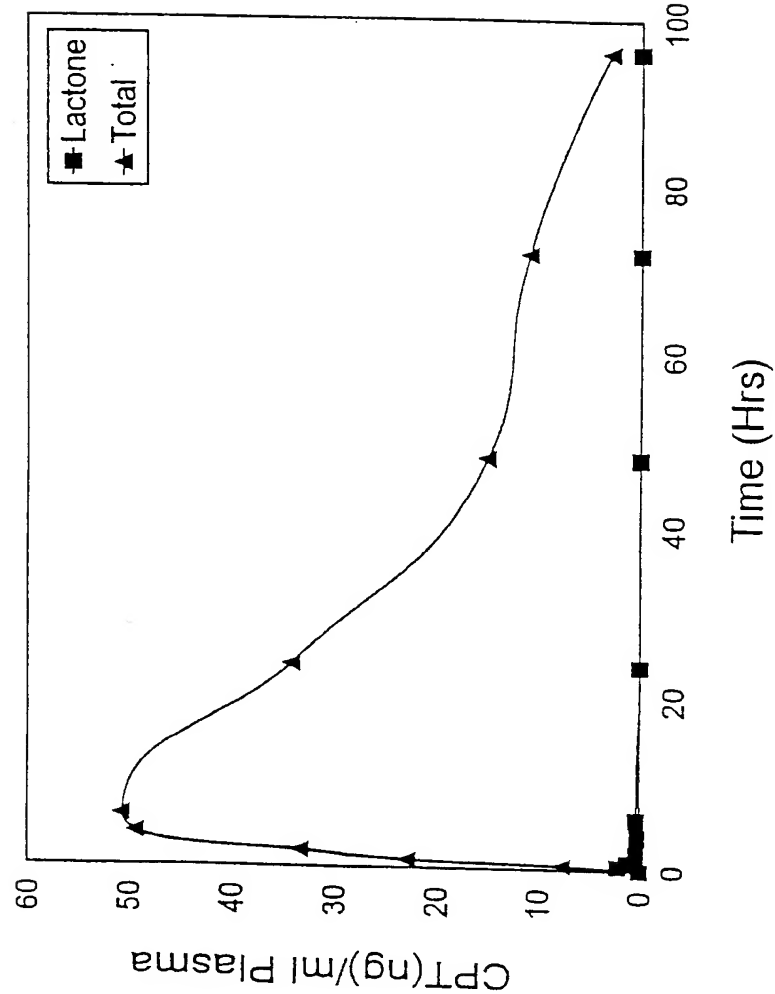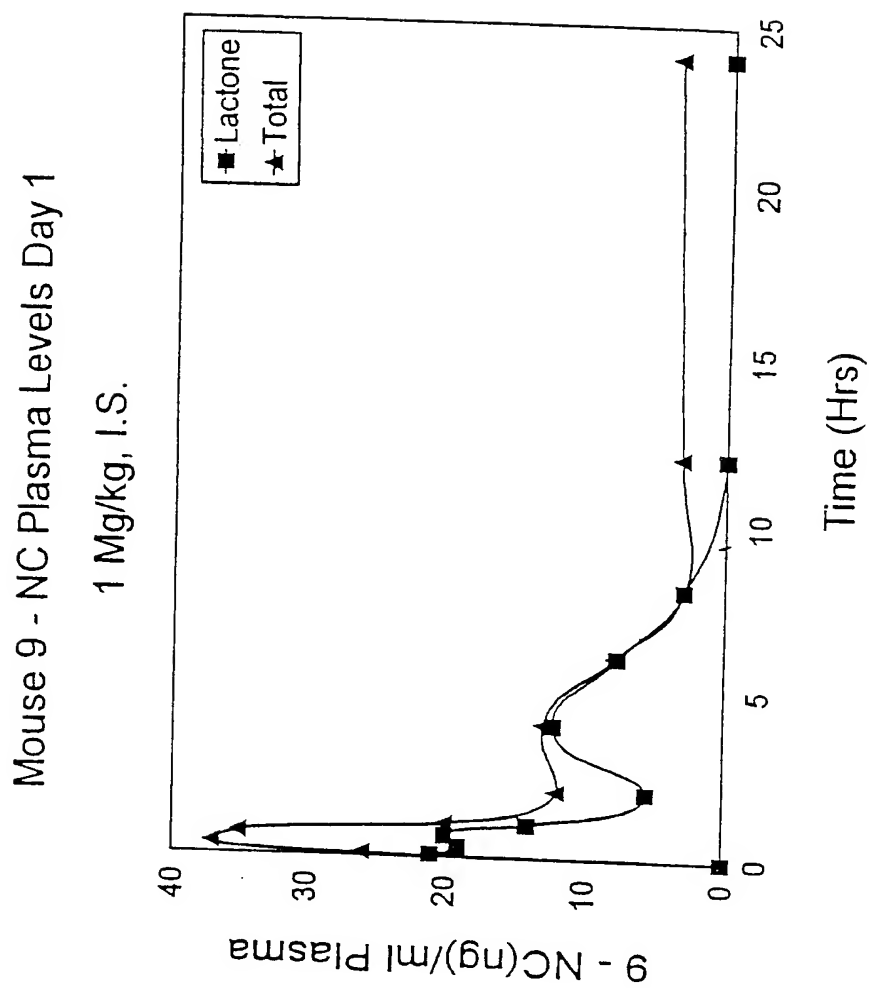
FIG. 3
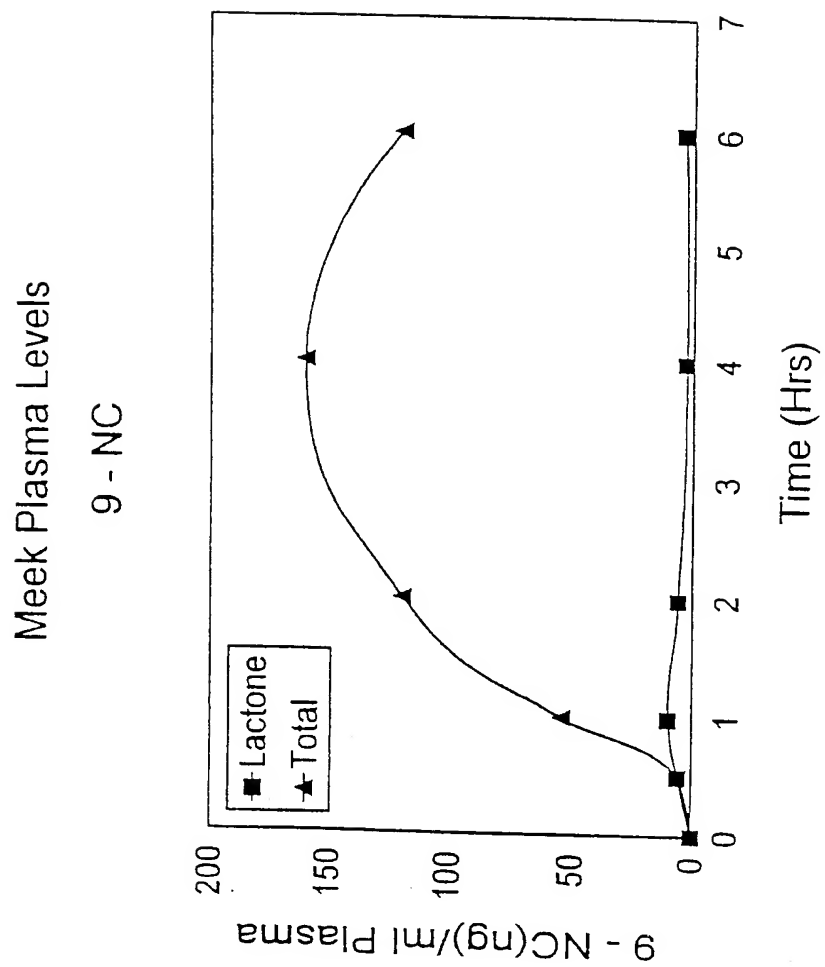
FIG. 4
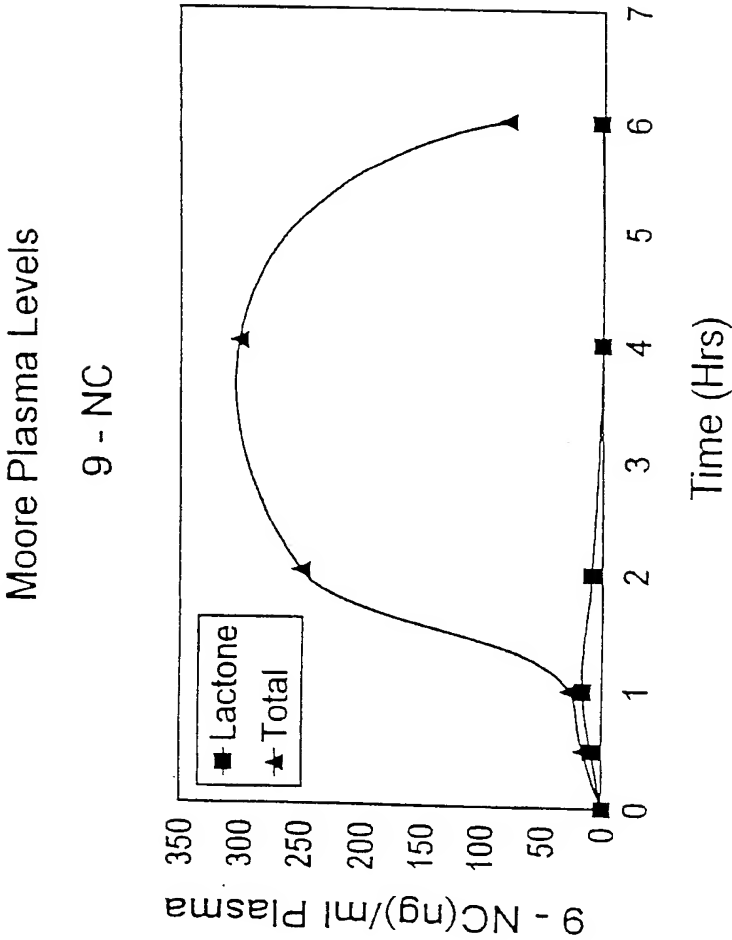
FIG. 5

The Activity of Prodrug 2 against
Human Breast Carcinoma

Time (days)

Tumor Size (mm3 x 1000)

Control
8 mg / kg
7 mg / kg
6 mg / kg
5 mg / kg
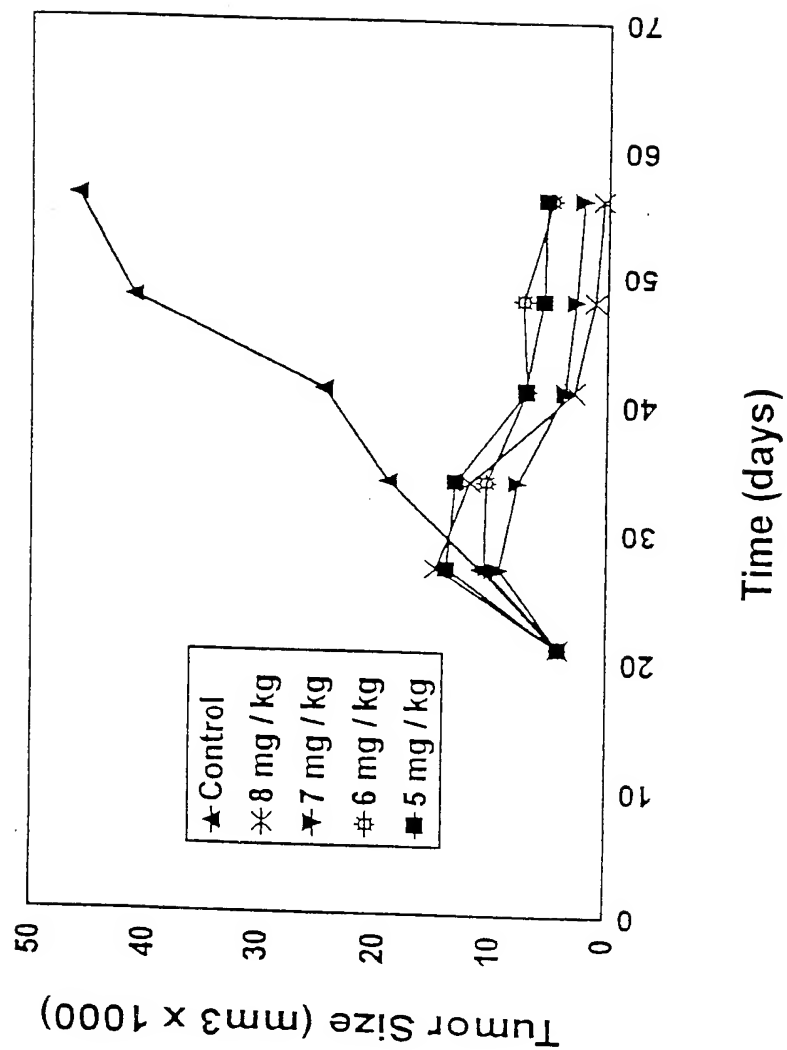
FIG. 6

Prodrug 2 in Human Plasma

4 Mg, P.O.

FIG. 7

Prodrug 11 in Human Plasma
10 Mg, P.O.
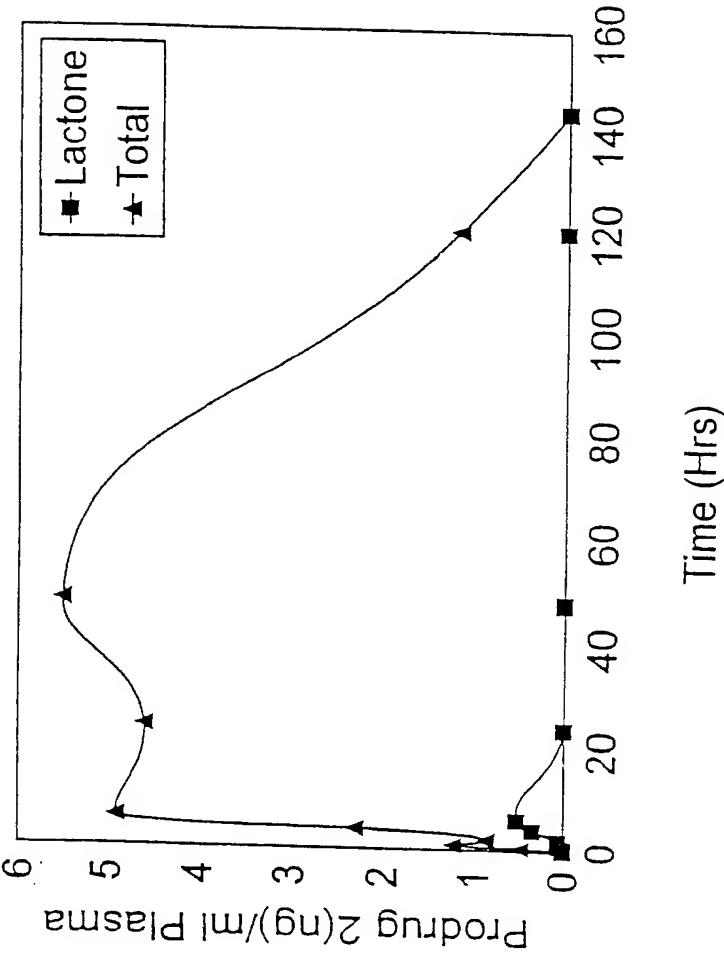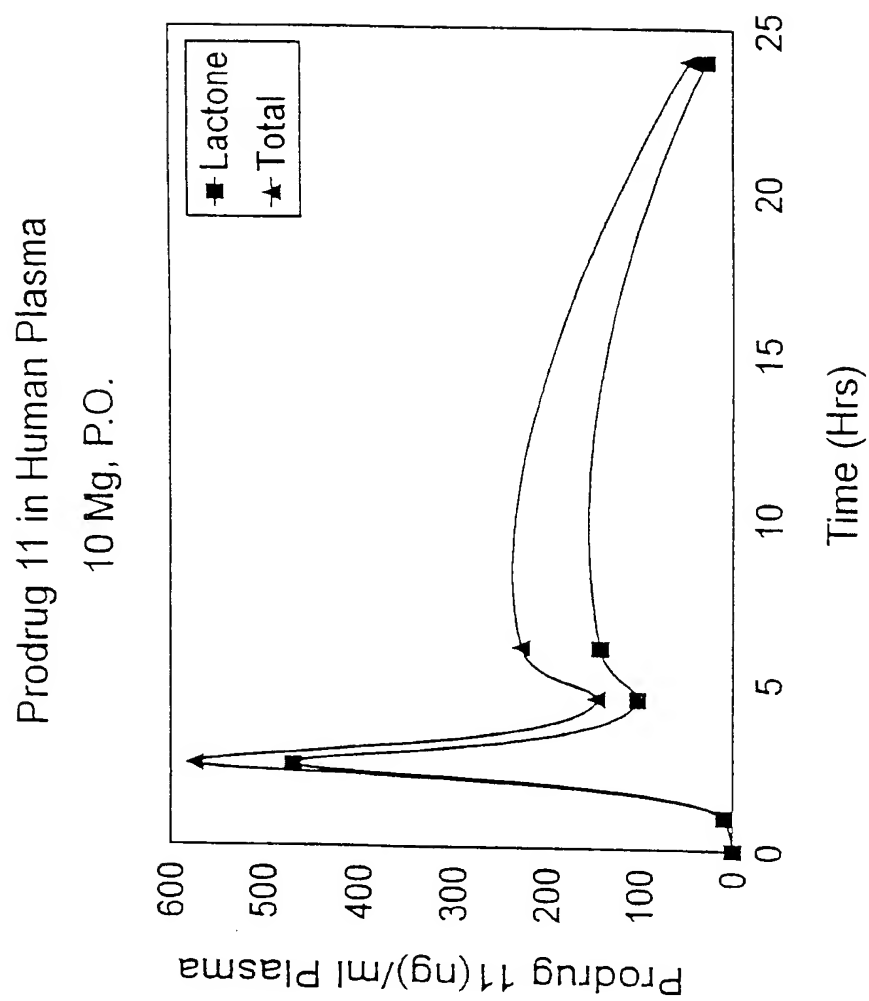
FIG. 8

FIG. 9

SC1087 - TUMOR SIZE
SW48 - COLON

FIG. 10

SC1067 - TUMOR SIZE
BRO - MELANOMA
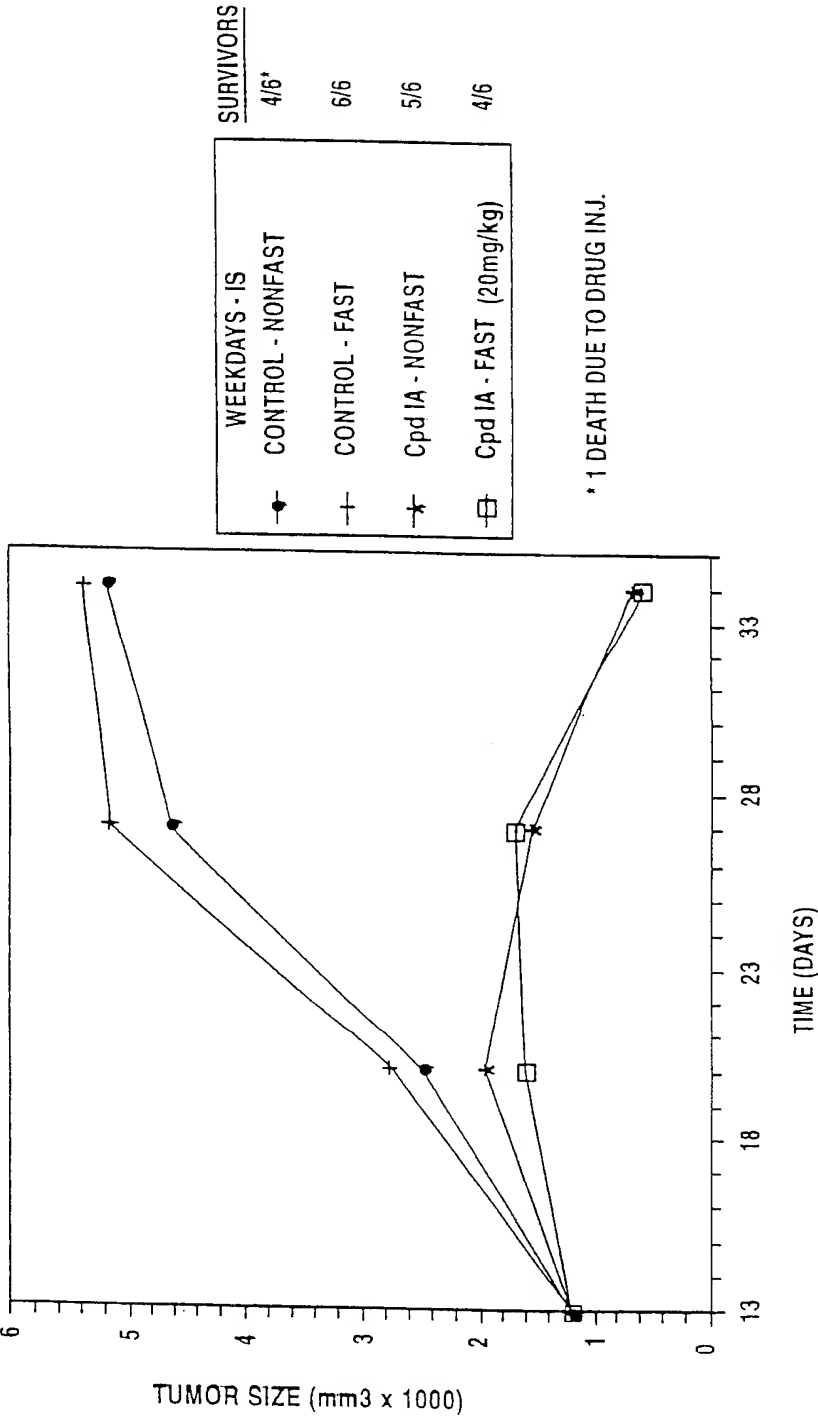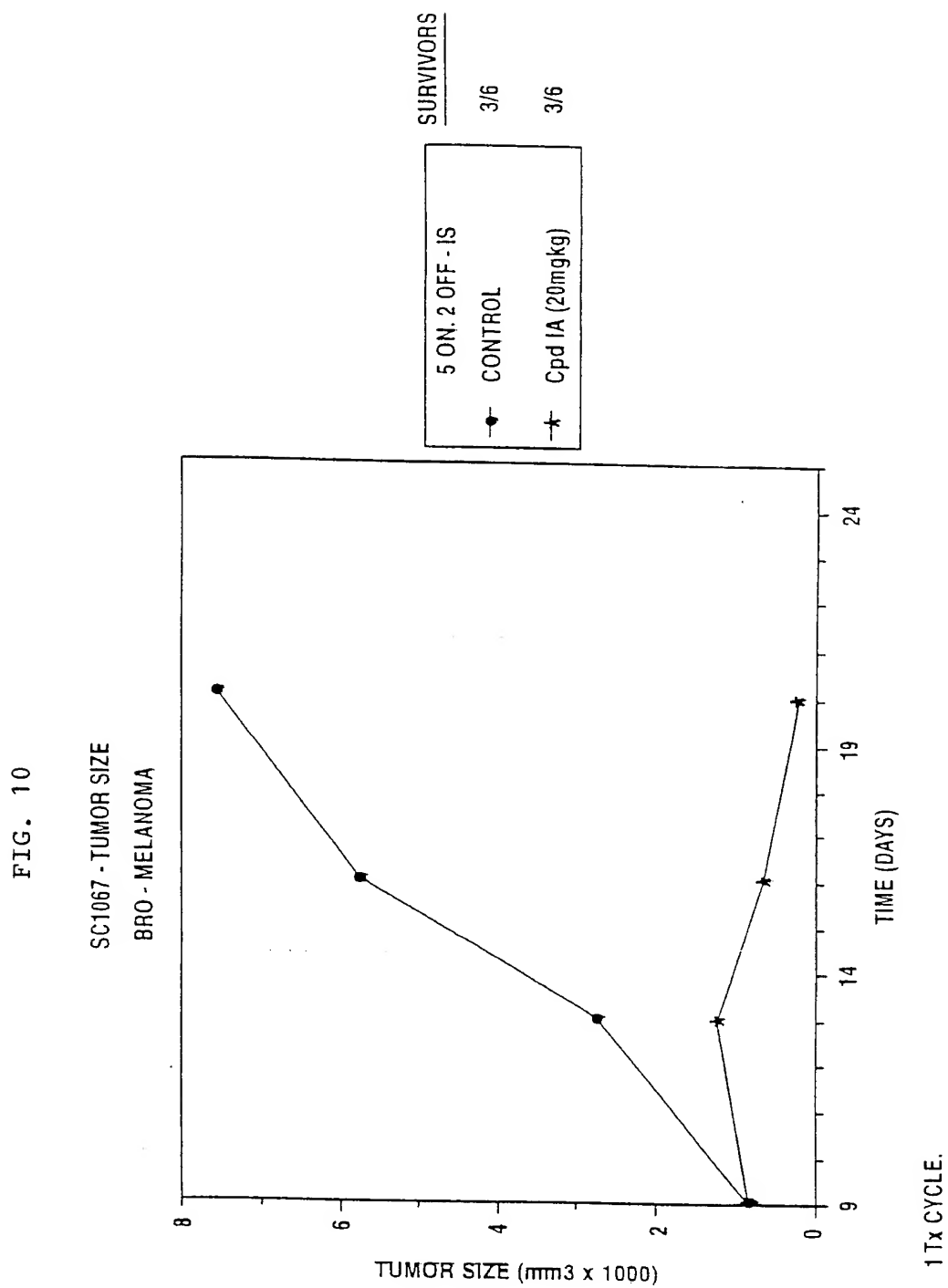
FIG. 11

SC1069 - TUMOR SIZE
BRE - STOMACH
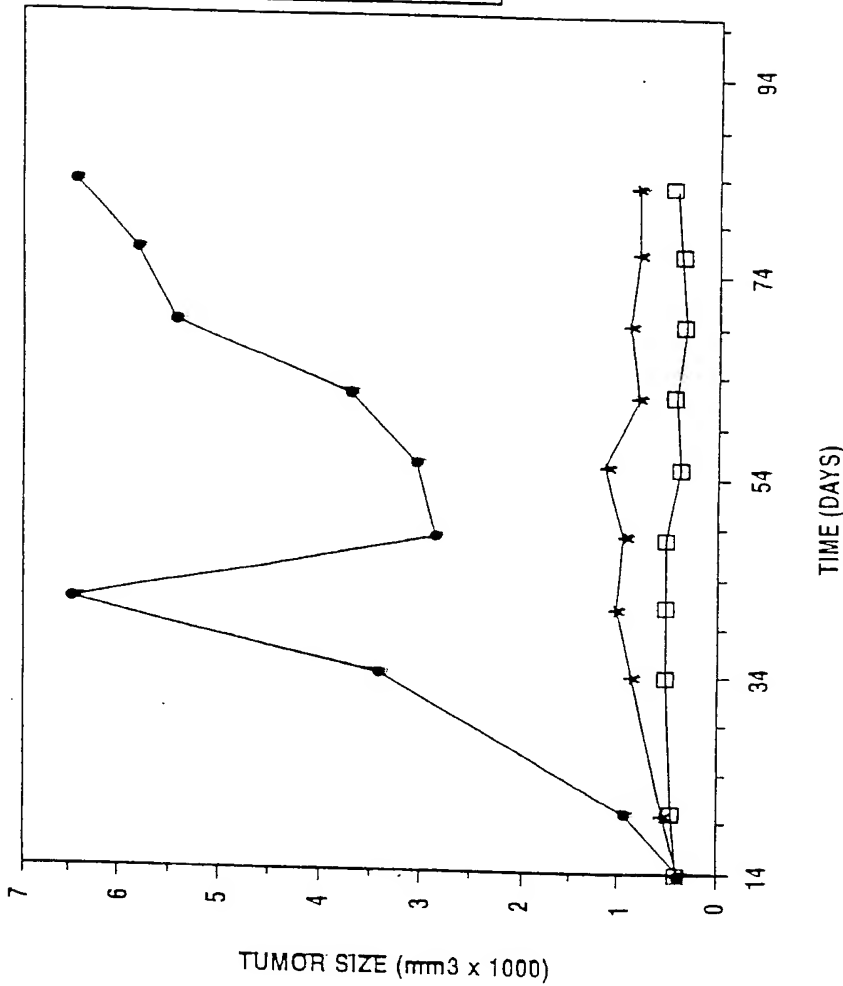
FIG. 12

SC1122 - TUMOR SIZE
DOY - LUNG Ca
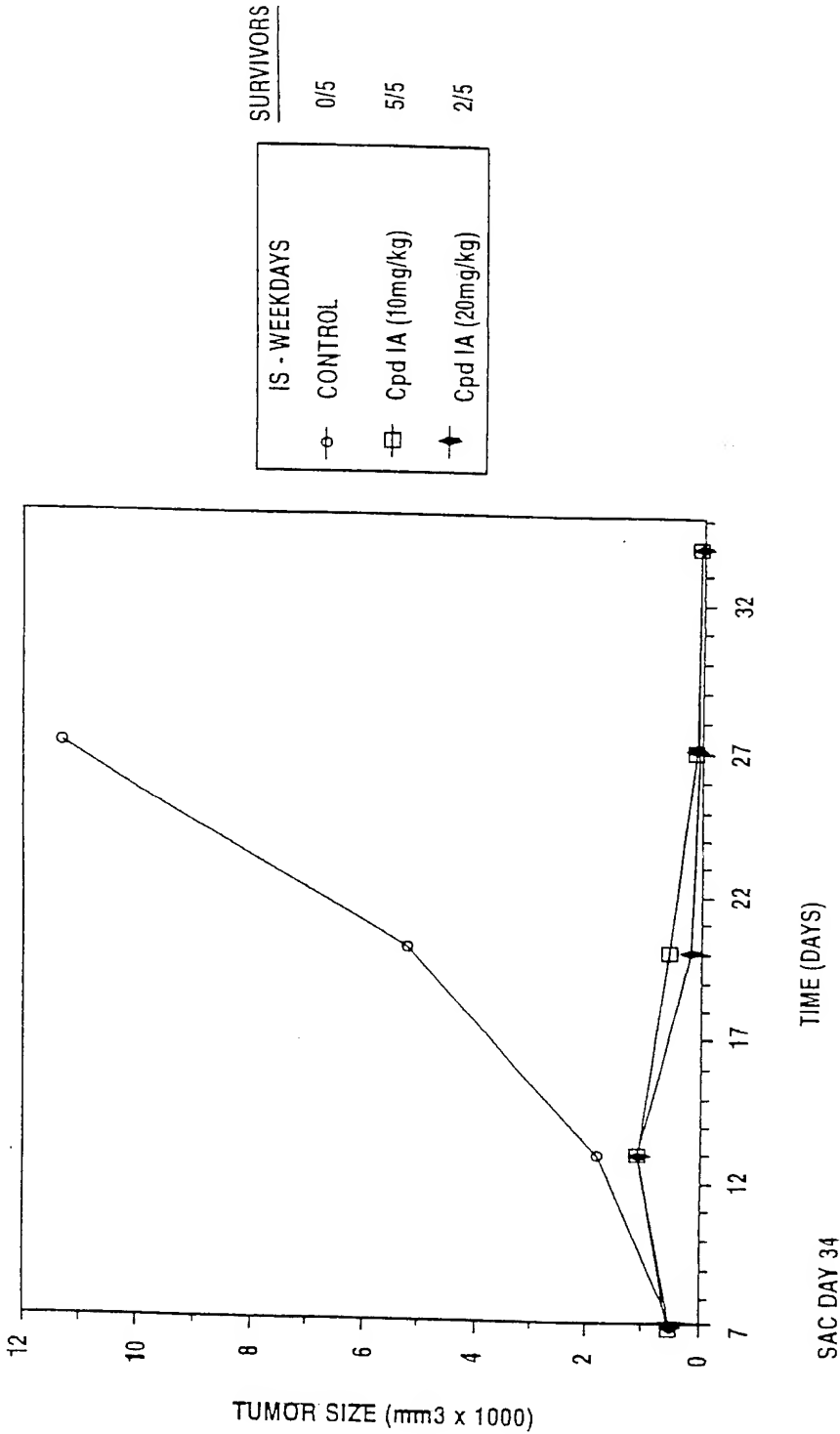
FIG. 13
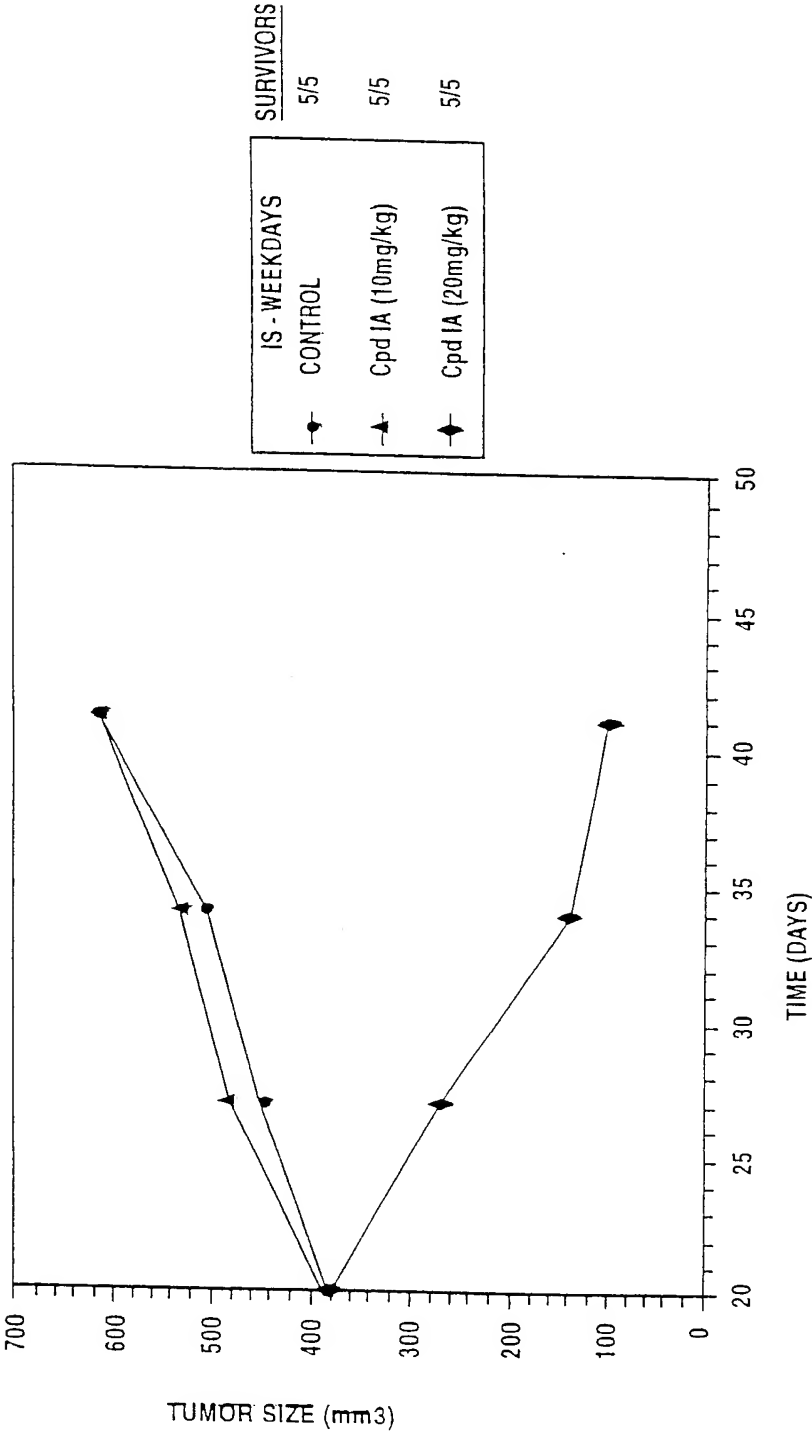
FIG. 14

SC1126 - TUMOR SIZE
McC - COLON Ca.
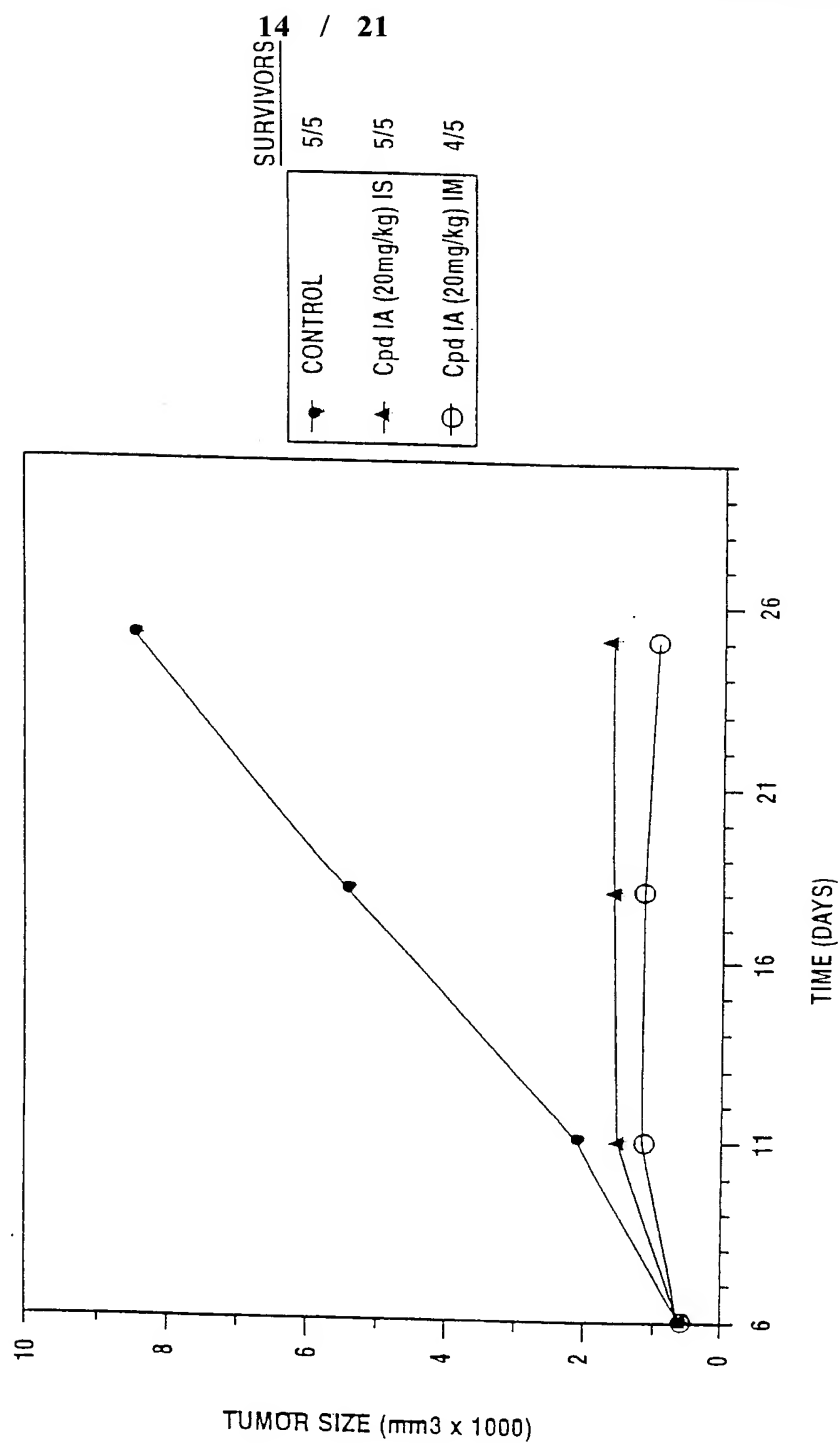
FIG. 15

SC1105 - TUMOR SIZE
SQU - COLON



SURVIVORS

| WEEKDAYS | | |
|---|---|---|
| CONTROL | ¢ | 0/6 |
| Cpd IA (10mg/kg) IM | ⊡ | 6/6 |
| Cpd IA (10mg/kg) IS | ◆ | 3/6 |

TUMOR SIZE (mm3 x 1000)

TIME (DAYS)

SAC DAY 111
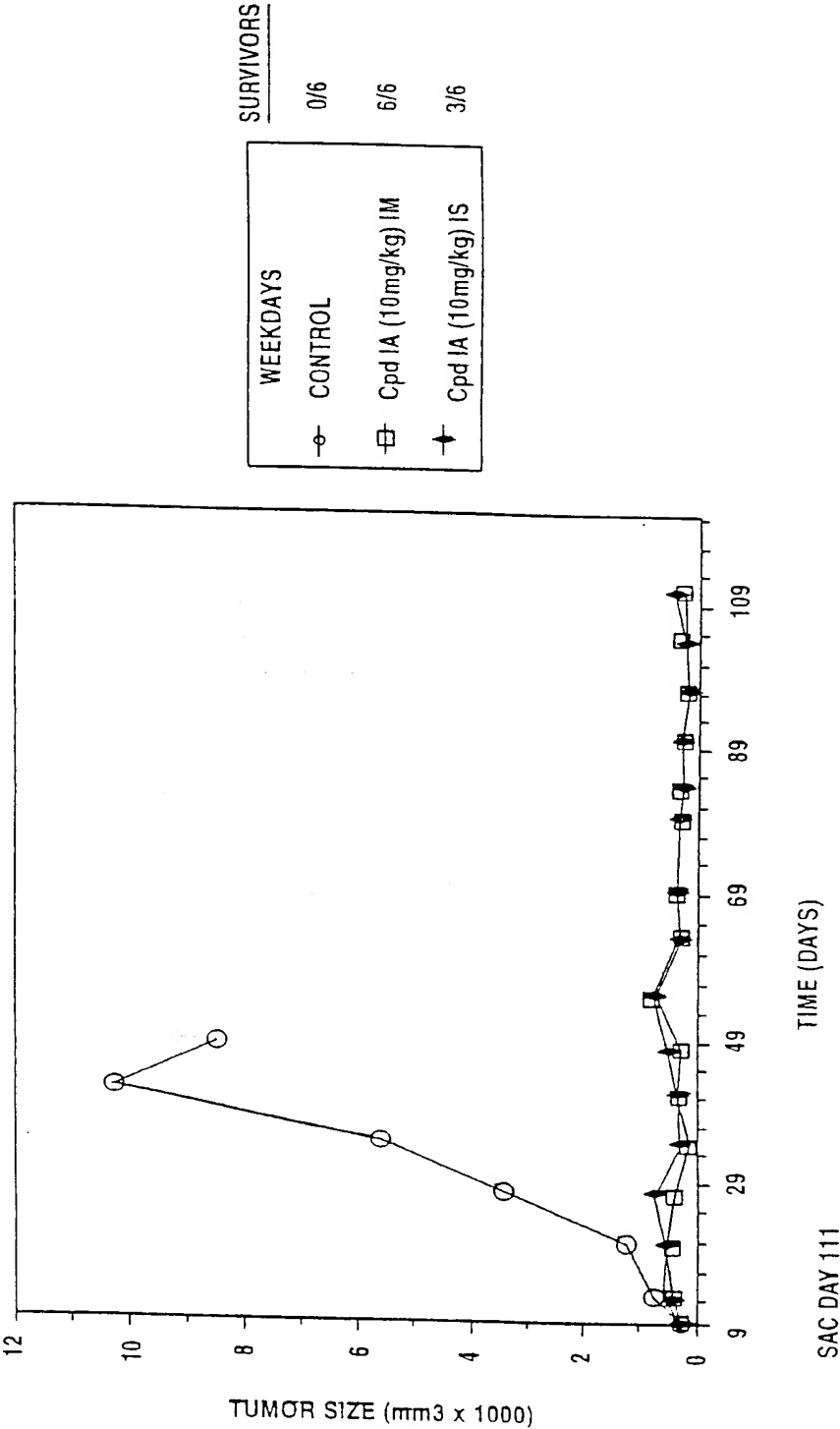
FIG. 16

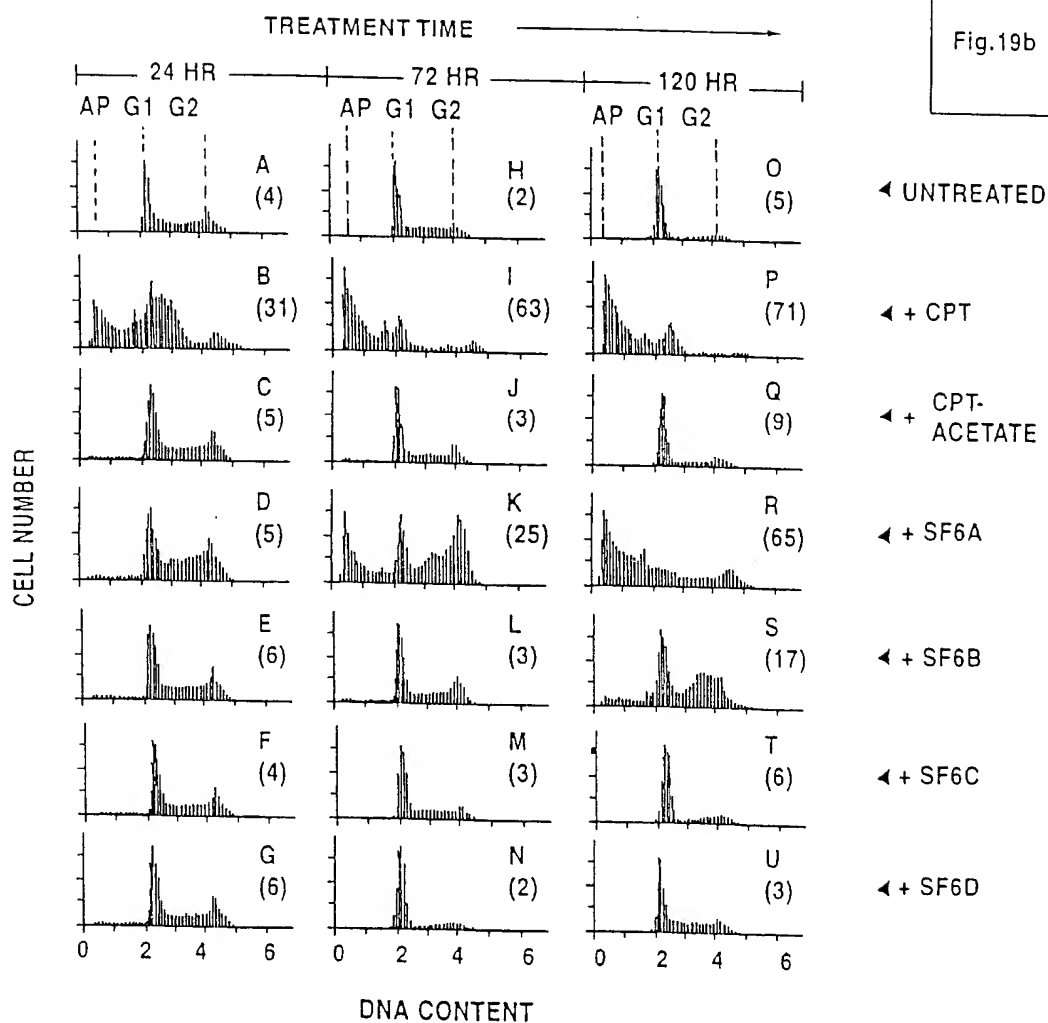SC1017 - TUMOR SIZE
CLO - BREAST

FIG. 17



A.

B.
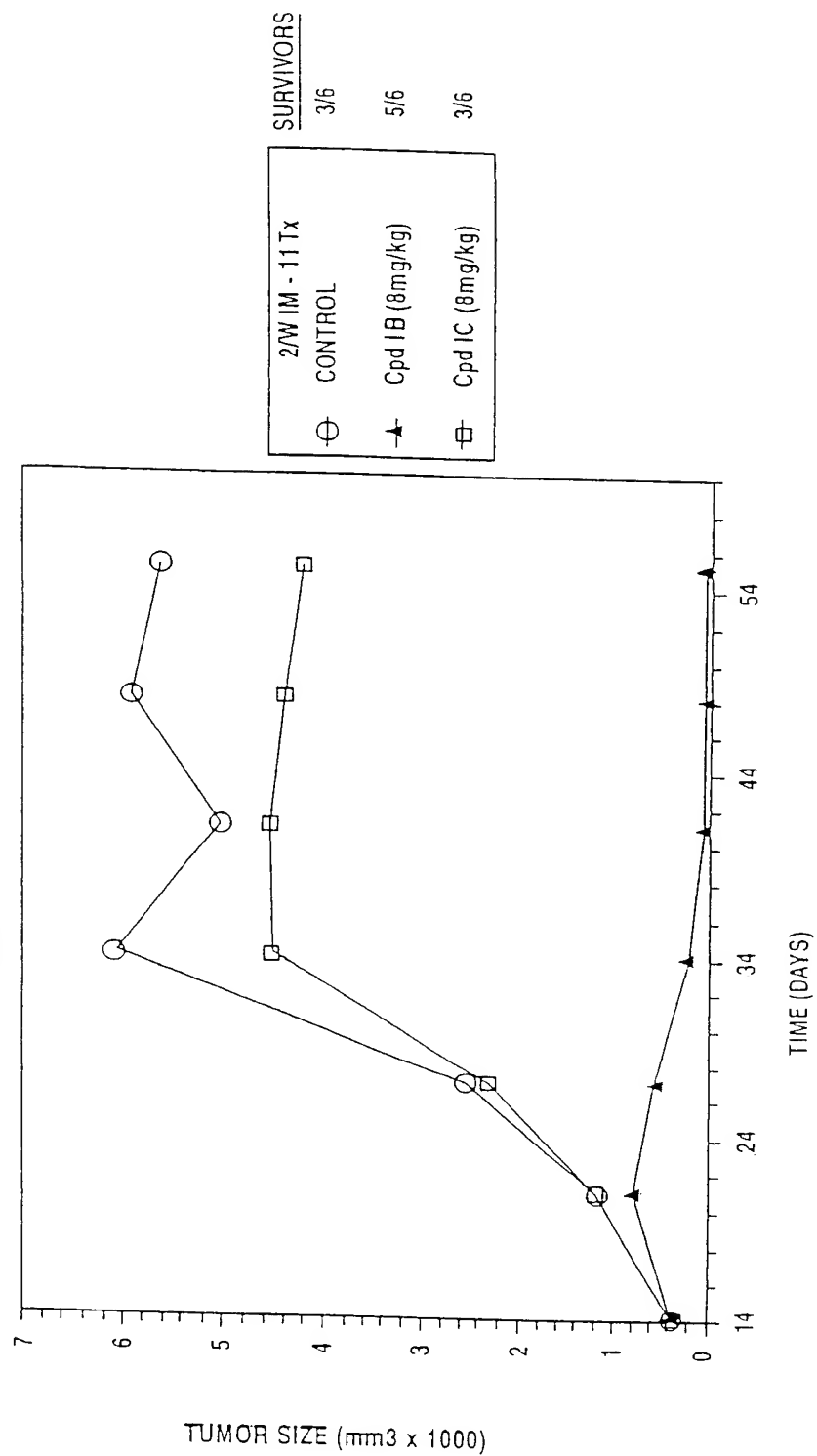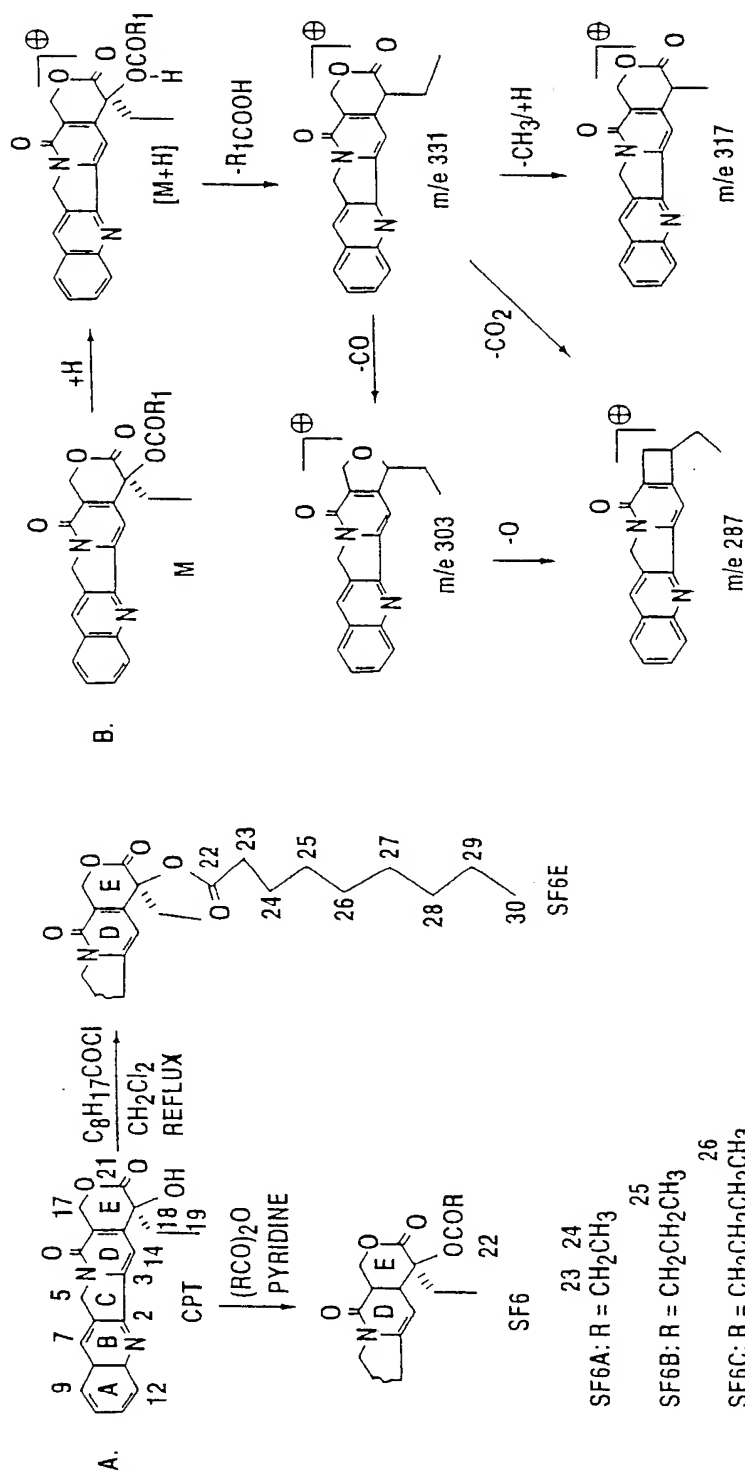
# Fig.18
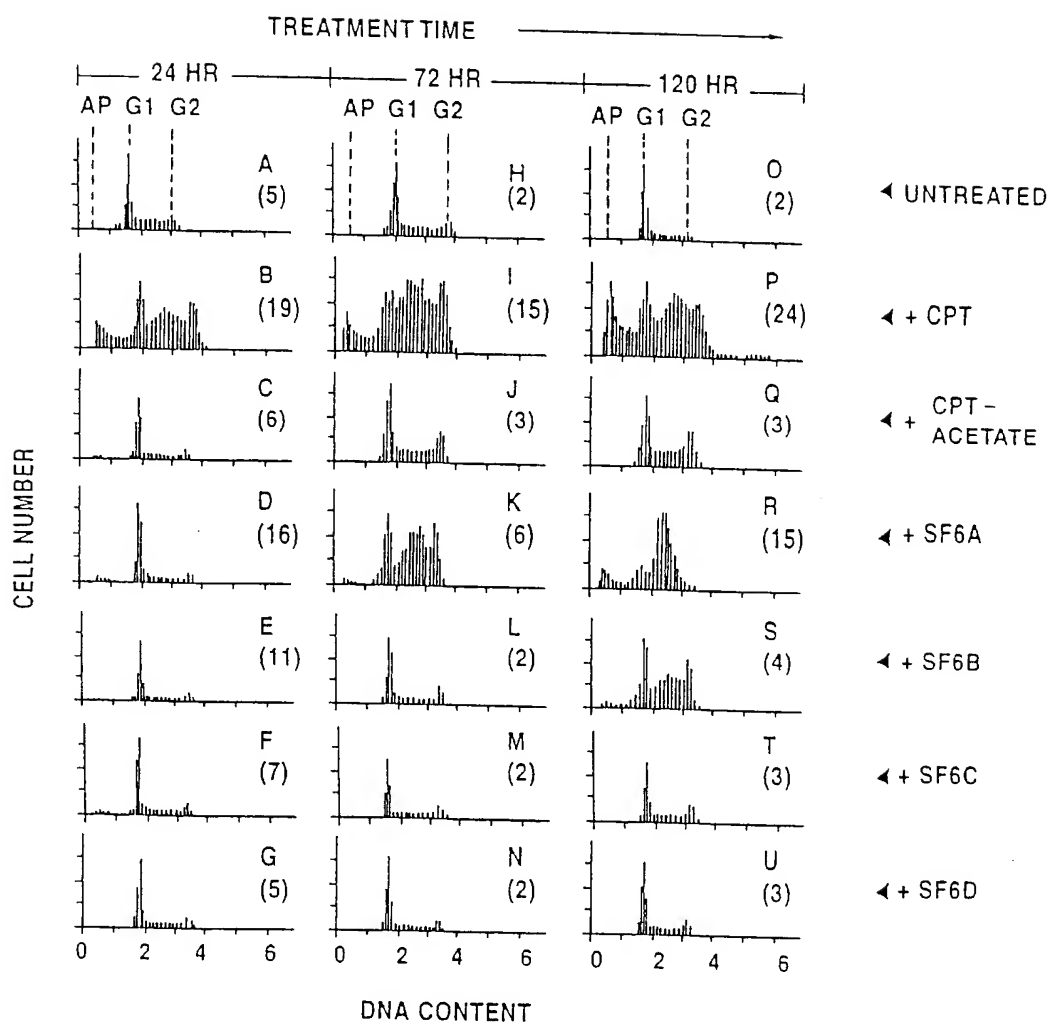
Fig.19a

Fig.19

| Fig.19a |
| Fig.19b |

# Fig.19b

# Fig.20